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463 POLIOMYELITIS VIRUSES IN TISSUE CULTURE *Joseph L. Melnick, Manuel Ramos-Alvarez, Francis L. Black, Anthony J. Girardi, and Daizo Nagaki*

486 PROTEOLYTIC ACTIVITY OF DOG BLOOD *Sidney N. Poly and Daniel L. Kline*

491 THE SIGNIFICANCE OF FEVER IN INFECTIONS *Ivan L. Bennett, Jr.*

505 INDIVIDUALIZATION OF CLERKSHIP TEACHING *Morris Green and Robert E. Cooke*

512 HEPATITIS IN URBAN POPULATION GROUP *S. J. Liau, F. P. Berg, and R. J. Bouchard*

527 PERCENTAGE OF TAPERED ENDS IN COLLAGEN *Madeline K. Keech*

536 EVENTS

543 BOOK REVIEWS

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CONTENTS

465 **POLIOMYELITIS VIRUSES IN TISSUE CULTURE. VII. EXPERIENCES WITH VIRAL AND SEROLOGICAL DIAGNOSTIC PROCEDURES** *Joseph L. Melnick, Manuel Ramos-Alvarez, Francis L. Black, Anthony J. Girardi, and Daizo Nagaki*

486 **THE SPONTANEOUS PROTEOLYTIC ACTIVITY OF DOG BLOOD** *Sidney N. Paly and Daniel L. Kline*

491 **THE SIGNIFICANCE OF FEVER IN INFECTIONS** *Ivan L. Bennett, Jr.*

506 **INDIVIDUALIZATION OF TEACHING ON THE PEDIATRIC CLERKSHIP AT YALE** *Morris Green and Robert E. Cooke*

512 **EPIDEMIOLOGY OF INFECTIOUS HEPATITIS IN AN URBAN POPULATION GROUP. REPORT ON AN EPIDEMIC IN A MEDIUM-RENTAL HOUSING PROJECT IN NEW HAVEN, CONNECTICUT IN 1953** *S. J. Liao, F. P. Berg, and R. J. Bouchard*

527 **THE PERCENTAGE OF TAPERED FIBRIL ENDS IN SKIN COLLAGEN FROM CASES WITH AND WITHOUT "COLLAGEN DISEASE"** *Madeline K. Keech*

536 **EVENTS**

545 **BOOK REVIEWS**

i **INDEX TO VOLUME 26**

THE YALE JOURNAL
OF BIOLOGY AND MEDICINE

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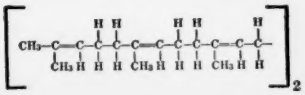

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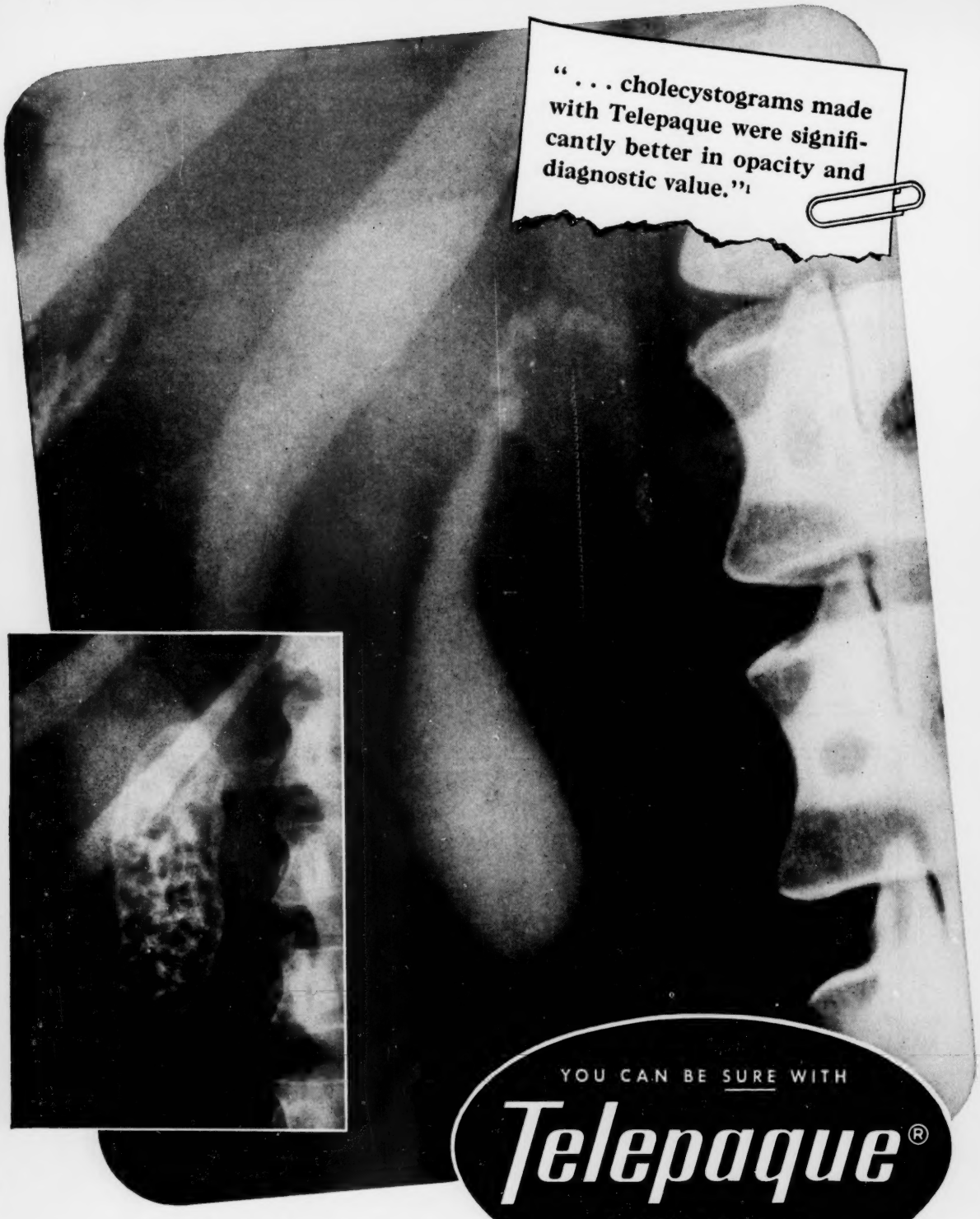

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POLIOMYELITIS VIRUSES IN TISSUE CULTURE

VII. EXPERIENCES WITH VIRAL AND SEROLOGICAL DIAGNOSTIC PROCEDURES[5]

Tissue culture inoculation has replaced the production of the experimental disease in the monkey as the method of choice for the direct isolation and antigenic identification of virus from materials obtained from poliomyelitis patients.[4, 11, 12, 16, 18] In addition, tissue-culture methods have now made practical serologic tests for antibody development in man after natural poliomyelitis infection[7, 15] or after active immunization.[14] However, there has been no standardization of methods as yet, which is perhaps desirable in a field so new.

The present report is concerned with the further development of tissue-culture techniques, with a view to their possible effectiveness in a poliomyelitis diagnostic laboratory. Three different lines of investigation have been pursued:

1. A comparison of the sensitivity and practicality of different methods for virus isolation.

2. An integration of the data obtainable from clinical specimens both by virus isolation and by serological tests for complement-fixing and neutralizing antibodies.

3. An investigation of possible correlations between the type of disease, the type of virus involved, and the immunological response.

In the early studies reported in the literature, cultures of human tissues or monkey testis were chiefly employed. Another monkey tissue which is readily responsive to poliomyelitis virus infection is the kid-

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[1] Fellow of the Behring Institute, Centro de Estudios Sobre Poliomielitis, Mexico City, 1953.
[2] Fellow of the Rockefeller Foundation, 1953-54, on leave from The Laboratory of Hygiene, Ottawa, Ontario.
[3] U. S. Public Health Service Fellow, 1952-53, on leave from the Virus Laboratory, Department of Pediatrics, University of Pennsylvania, Philadelphia.
[4] Fellow of the China Medical Board, 1953, on leave from Kitasato Institute, Tokyo.
[5] Aided by a grant from the National Foundation for Infantile Paralysis.
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ney,[5, 9, 14, 17] and in the present study, cultures of this tissue have been chiefly used. Not only can considerably more tissue be obtained from a single animal than is the case with testis, but kidney tissue and the epithelial cells which grow out from it are usually more responsive to poliomyelitis virus infection than testicular tissue and its outgrowth of fibroblasts. A further advantage is the greater rapidity of the cytopathic changes in kidney cultures, which has already been noted by others.

COLLECTION OF SPECIMENS

In order to broaden the study, materials from patients within two representative areas were studied: (a) New Haven, Connecticut, where in 1952 the incidence of poliomyelitis was low and (b) Lock Haven, Pennsylvania, where a severe poliomyelitis outbreak occurred in the same year. In addition, two series of tests for poliomyelitis virus were carried out on the feces of chimpanzees fed the W-S Type I strain.

*Poliomyelitis in New Haven, Conn., 1952.** In 1952, of 59 cases in New Haven County (11 per 100,000), 37 were reported as paralytic and 22 as nonparalytic cases. The most recent outbreak of severe proportions in this locality took place in 1943.

*Poliomyelitis in Lock Haven, Pa., 1952.** This was the first large epidemic to be reported in recent years in this town and its surrounding area, Centre and Clinton counties; 104 cases were reported, the majority being paralytic. The over-all case rate for the two counties was 120 per 100,000. The case rate for those under 15 years of age was 340 per 100,000 and for those 15 years and over, 32 per 100,000.

Experimentally infected chimpanzees. In addition to the human specimens, feces were collected from eight chimpanzees fed the W-S Type I poliomyelitis virus; none of the animals developed signs of illness. The feces were collected almost daily for about one month after the virus feeding. Specimens from each animal were made into eight to ten pools, each representing a sample of two to three days. They were then treated as described below in the same manner as the human specimens.

Stools were collected chiefly during the first week following hospitalization of patients. One to three specimens were taken from each patient and stored at —20° C. In some instances they were refrigerated at 4° C., until

* We are indebted to the medical and pediatric staffs of the Grace-New Haven Community Hospital and to Drs. Gerald McDonough, Edward Rabe, and Merl Colvin, for their assistance in the collection of materials from the New Haven and Lock Haven areas, respectively.

suspension of the material could be prepared, but in no case were the samples allowed to remain at this temperature for more than 72 hours. Suspensions were prepared with single or pooled samples, in final concentration of 20%, by overnight shaking in sterile distilled water at 4° C. This was done in 250 ml. centrifuge bottles which could then be spun at 2,500 rpm. for 20 minutes to remove gross particulate matter. The supernatant fluids were stored at —20° C. prior to virus isolation studies.

Just prior to the inoculation of the tissue cultures, the 20% stool suspension was thawed rapidly, mixed, and centrifuged at 3,000 rpm. for 30 minutes. (For some experiments an aliquot was spun at 18,000 rpm. for 30 minutes). The supernatant fluid was removed and penicillin, plus streptomycin, added to this material to yield final concentrations of 500 to 2,000 units and of 0.5 to 2 mg. per ml., respectively. The mixture was allowed to stand for 10 minutes before inoculation into cultures.

Throat swabs were collected only from the New Haven patients, also during the first few days of hospitalization. They were stored at —20° C. in 1.0 ml. of balanced salt solution containing penicillin and streptomycin. After thawing, the swab was squeezed and the expressed fluid was added to the fluid in which it had been frozen.

Serum samples were collected from many of the patients who provided stool specimens. An attempt was made to collect the first serum early after admission to the hospital, a second sample two to three weeks later, and a third sample about six weeks later. They were also stored at —20° C. until used.

VIRUS ISOLATION METHODS

Methods for the isolation of virus using two types of tissue culture, suspended cell and outgrowth cell cultures, have been compared. *Suspended cell cultures* consisted of about 15 fragments of monkey kidney (1 to 2 mm. in diameter) bathed in 1 ml. of nutrient medium in a 16 x 150 mm. test tube. These cultures were usually used on the day of preparation. *Outgrowth cell cultures* were obtained by attaching fragments of kidney or testis tissue to the wall of the test tube by means of a plasma clot or by prewarming the tube.[6, 8, 9] One ml. of medium was added to each tube and the tubes were incubated for a period of one or two weeks, to allow a sheet of cells to grow out from the explants. The medium used for both types of culture was either mixture #199,[10] or a mixture containing 0.5% lactalbumin hydrolysate, 20% bovine serum ultrafiltrate, and 79.5% Earle's balanced salt solution.[8] All tubes were placed at a slightly inclined position

so that a thin layer of fluid was present over the tissue fragments, and held stationary during incubation at 36° C.*

Isolations from throat swabs were carried out in suspended cell cultures in the same manner as the stool isolations (see below).

Isolation of virus in suspended cell cultures. One-tenth ml. of the penicillin-streptomycin-treated stool suspension was added to each of two suspended cell cultures. The tubes were then held stationary or placed in a roller drum at 36° C. for five days,** when the fluids were harvested. A neutralization test (see below) could be carried out on these fluids directly to determine whether an agent had been isolated, and, if so, its type. However, when there was no urgency, it was preferable to pass the agent once on outgrowth cell cultures to be certain that a cytopathogenic agent had been isolated before making the neutralization test. For this passage 0.1 ml. of undiluted fluid harvested from the suspended cell culture was used as the inoculum. The passage tubes were then observed for cytopathic changes, and, if present, the virus was identified in a third passage by neutralization with type-specific antisera. With suspended cell cultures, the cytotoxicity of stools (which in outgrowth cell cultures may interfere with observation of specific, viral cytopathic changes) was never a problem. However, blind passage of the culture fluid was required before the presence of virus could be established.

Isolation of virus in outgrowth cell cultures. To each of four culture tubes showing outgrowth of cells, 0.1 ml. of the treated stool suspension (or throat swab extract) was added, and the tube was held stationary at 36° C. Tubes were examined daily for ten days. If the stools were toxic for cultures it was usually apparent within 24 hours, whereas degeneration caused by a cytopathogenic agent did not usually become apparent until between the third and fifth days. If stools were toxic, fluids for passage were harvested on the fifth day, otherwise they were harvested when degeneration was clearly evident. Passage and neutralization tests were then carried out.

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* More recently virus isolations have been made with monolayer cultures prepared from trypsinized monkey kidneys according to Youngner (Proc. Soc. Exp. Biol., N. Y., 1954, *85*, 202-205). We have found that mixture #199 is not essential and that the cultures grow out in monolayers ready for use in three to four days when the trypsinized cells are dispensed in the following medium: 0.5% lactalbumin enzymatic hydrolysate; 2.0% calf serum (from animals about six months of age); and 97.5% Hanks' balanced salt solution.

** A series of experiments with stools of patients and with established strains of poliomyelitis virus showed that virus is adsorbed in suspended cell cultures in about an hour and then multiplies in them to reach peak titers usually in five days. During the period of viral multiplication, the toxic factor in stools becomes inactivated (or perhaps adsorbed to the tissue fragments). The number of isolations can be increased by about 10 per cent if harvests are also made on the 10th and 15th days.

Identification of viral types by neutralization tests. Fluids from primary isolation in kidney tubes, or from second passage cultures showing a viral type of degeneration, were tested in outgrowth cell cultures against each of the three type-specific poliomyelitis antisera. The sera were prepared by hyperimmunization of monkeys and had titers of over 1:1,000 against 100 TC_{50} doses of virus. The background for establishing the conditions of the test as used in this laboratory has been reported in detail elsewhere.[8] In brief, 0.2 ml. of undiluted TC (tissue culture) fluid was mixed with

TABLE 1. COMPARISON OF TITERS OF STOOLS IN OUTGROWTH CELL CULTURES WITH THE NUMBERS FOUND POSITIVE FOR VIRUS IN SUSPENDED CELL CULTURES

Titer in outgrowth cell cultures	*No. of specimens*	*Results in suspended cultures*	
		Positive	*Negative*
Neg.	42	0	42
10^{-1}	34	14	20
10^{-2}	6	4	2
10^{-3}	2	2	0
Total	84	20	64

0.2 ml. of each antiserum, previously diluted fivefold with balanced salt solution. After incubation for one hour at room temperature, 0.1 ml. of each serum-virus mixture was inoculated into two tissue culture tubes. The cultures were incubated in a stationary position until read, three and six days later for kidney tissue, four and eight days later for testicular tissue. The virus was identified by the serum which neutralized its cytopathogenic property. If a virus was not neutralized by any of the three antisera, it was tested again by carrying out titrations of the virus in the presence of each of the antisera. Occasionally a poliomyelitis virus of high titer may not be neutralized if undiluted TC fluid is mixed with 1:5 antiserum.

Comparison of the sensitivity of suspended cell cultures with that of outgrowth cell cultures for isolation of virus. These experiments were conducted with 70 fecal samples obtained from 8 chimpanzees fed poliomyelitis virus and with 14 specimens from human cases of poliomyelitis. The specimens were run by the two techniques outlined above. Of the 84 specimens, 20 were positive by the suspended cell method, but 42 yielded virus in cultures of outgrowth cells. As shown in Table 1, the concentration of virus in

the stool specimens had an important bearing on these findings. Titrations of the positive stools in cultures of epithelial outgrowth yielded values of about 10^{-1} for most of the specimens. Only eight samples gave higher values and of these, six were found to have yielded virus in the suspended culture technique. The two samples which titrated to 10^{-3} were positive by both methods.

SEROLOGICAL METHODS

Determination of neutralizing antibodies. Sera were tested for the presence of neutralizing antibodies against the three types of poliomyelitis virus. The application of the TC neutralization test to human sera has been reported.[5, 7, 14, 15] In the present study the Brunhilde, Y-SK, and Leon TC-adapted strains were used as representatives of Types I, II, and III viruses, respectively, at concentrations representing 100 TC_{50} doses of each virus. Readings were made on the third to fourth day for kidney tubes, and on the fourth to sixth day for testicular culture tubes. The results reported are those obtained when the control titration, carried out simultaneously with the test, indicated that the dose of virus used was equivalent to 100 TC_{50}.[5] A number of uninoculated control tubes, and tubes inoculated with the serum alone, were present in each test to rule out nonspecific degeneration.

Two dilutions, 1 : 5 and 1 : 50 (or 3 dilutions, 1 : 5, 1 : 20, 1 : 80), of each serum were added, in 0.2 ml. volumes to equal volumes of suspensions of each type of virus containing 200 TC_{50} doses per 0.1 ml. Thus after mixing, the final concentrations of serum were 1 : 10 and 1 : 100 (or 1 : 10, 1 : 40, and 1 : 160), and of virus, 100 TC_{50} doses per 0.1 ml. After the mixture had stood for 1 hour at room temperature, 0.1 ml. of each serum-virus mixture was inoculated into each of two outgrowth cell culture tubes.

Determination of complement-fixing antibodies. The procedure used in this laboratory for C-F determination in poliomyelitis studies has been described elsewhere.[1] By carrying out each titration on plates at three low concentrations of complement, it was generally found possible to use unconcentrated infected tissue culture fluids as antigens and to avoid the concentration procedure recommended by Svedmyr, Enders, and Holloway.[15] The Brunhilde, Y-SK, and Leon strains of virus were used for the production of types I, II, and III antigens, respectively. Unless otherwise stated the results reported in this paper were obtained by using unconcentrated antigens. It was found that many heterotypic reactions could be eliminated at the low concentration of complement and antigen.

RESULTS

STUDIES ON CLINICAL SPECIMENS FROM NEW HAVEN DURING A YEAR OF LOW INCIDENCE OF THE DISEASE

Of 42 *stool specimens* examined from 34 patients, 19 (from 18 patients) were positive for poliomyelitis virus when suspended monkey kidney tissue cultures were employed for primary isolation attempts. Identification of virus type by TC neutralization tests indicated that viruses of

TABLE 2. DISTRIBUTION OF VIRUS TYPES AMONG CASES DIAGNOSED AS PARALYTIC AND NONPARALYTIC POLIOMYELITIS IN AN EPIDEMIC AND A NONEPIDEMIC AREA, 1952

		Per cent positive samples				
NEW HAVEN	*No.*	*Total positive*	*Type 1*	*2*	*3*	*Virus not isolated, %*
Paralytic cases	11	64	18*	18*	27*	36
Nonparalytic cases	23	48	13	26	9	52
LOCK HAVEN						
Paralytic cases	40	93	65	27	0	7
Nonparalytic cases	30	80	67	13	0	20

* Includes one bulbar case.

Case rate: New Haven area, 11 per 100,000
Lock Haven area, 120 per 100,000

all three types had been present in the New Haven area during the summer of 1952. In Table 2 the total number of cases is broken down into groups according to the nature of the illness involved and the type of virus isolated. Isolation attempts which gave negative results are also recorded. In view of the finding of all three types in approximately equal proportion, several of the stool specimens were run again, this time by another worker, and the same virus type was isolated from each specimen. Type I virus was found in 5 patients, aged 2, 3, 11, 25, and 29 years; Type II in 8, aged 1, 4, 4, 5, 6, 12, 15, and 18; and Type III in 5, aged 4, 9, 11, 13, and 14. Sixteen samples were negative. The three bulbar cases each yielded a different type of virus. The 8 paralytic cases yielded 4 positive specimens, two belonging to Type III and one to each of the other two types. The 23 nonparalytic patients yielded 11 isolates, 3 Type I, 6 Type II, and 2 Type III viruses. No viral type seemed to have a predilection for a particular age group.

Of 44 *throat swab specimens* examined, 7 were positive for poliomyelitis virus, with all 3 viral types among the 7 isolates. The nature of each case yielding virus in the throat, together with a comparison with the stool isolation attempts on these patients, is recorded in Table 3. The stools of 5 of the 7 patients had also been examined for poliomyelitis virus, and of these five stools, all but one yielded poliomyelitis virus. In each case virus

TABLE 3. ISOLATIONS OF POLIOMYELITIS VIRUSES FROM THROAT SWABS OF NEW HAVEN PATIENTS DIAGNOSED AS POLIOMYELITIS CASES

Type virus isolated from throat swab	*No. of patients*	*Nature of case*	*Virus isolation from stool*
I	2	Nonparalytic	Negative
		Nonparalytic	Type I
II	3	Nonparalytic	Specimen unavailable
		Nonparalytic	Type II
		Paralytic	Specimen unavailable
III	2	Paralytic	Type III
		Bulbar	Type III
Negative	37		
Total positive	7/44		

of the same type was isolated from the intestine and throat. The lower frequency of virus recoveries from the throat is in keeping with the results of previous experiments in which monkeys had been used as test animals.

STUDIES ON CLINICAL SPECIMENS FROM LOCK HAVEN DURING A YEAR OF HIGH INCIDENCE OF THE DISEASE

Virus isolations. Of 128 stool specimens taken from 70 patients, 90 from 61 patients were positive for virus. Thus 87% of the patients yielded virus. The following breakdown of the data was made (see Table 2):

1. Thirty-seven of 40 (93%) of the paralytic patients and 24 of 30 (80%) of the nonparalytic patients were positive.

2. Type I virus was recovered from 46 patients, of whom 26 (57%) were paralytic and 20 (43%) were nonparalytic.

TABLE 4. SEROLOGICAL FINDINGS ON 18 PATIENTS WITH PARALYTIC POLIOMYELITIS FROM WHOM TYPE I VIRUS WAS ISOLATED

Number of patient	*Age in years*	*Stool isolations*		*Serological findings*						
					Neutralizing antibody titer			*C.F. antibody titer*		
		Days after onset	*Type of virus*	*Days after onset*	*Type I*	*Type II*	*Type III*	*Type I*	*Type II*	*Type III*
28	0.2	6	I	5	100+	100+	0[1]	0[2]	0	16
		10	I	32	100+	100+	0	32[3]	0	8
65	0.3	5	I	4	40	10	0	0	0	0
				26	160+	40	0	4	0	0
				37	160+	160	0	32	0	0
135	1.4	8–9	neg.	7	100+	10	0	0	0	8
		12–13	I	25	100+	0	0	32	0	16
83	1.5	8	I	7	160+	20	0	0	0	16
		9–12	neg.	49	160+	40	0	32	0	4
				70	160+	160+	0	32	0	0
2	2	16	neg.	14	100+	0	0	0	0	0
		17	I	49	100+	10	0	0	0	0
				70	100+	10	0	16	0	0
101	2	9–10	I	4	100+	0	0	0	0	0
				21	100+	0	0	32	0	0
				44	100+	10	0	32	0	0
104	2	5–6	I	3	10	0	0	4	0	0
		7	I	36	100+	10	0	8	0	4
10	3	6	neg.	4	100+	32	0	0	0	0
		8	I	24	100+	0	0	0[4]	0	0
		9	I							
13	3	5	I	2	100+	0	0	0	0	0
				26	100+	0	0	32	0	0
				47	100+	0	0	32	0	0
82	3	9	I	6	160+	30	0	0	0	0
		11	I	28	160+	10	0	8	0	0
				41	160+	0	0	8	0	0
110	3	8	I	9	100+	32	0	0	0	0
				34	100+	0	0	16	0	0

[1] 0 = neutralizing titer of less than 10.
[2] 0 = C-F titer of less than 4.
[3] 32 = C-F titer of 32 or greater.
[4] Greater than 4 when concentrated antigen was used.

Number of patient	Age in years	Stool isolations: Days after onset	Stool isolations: Type of virus	Serological findings: Days after onset	Neutralizing antibody titer: Type I	Neutralizing antibody titer: Type II	Neutralizing antibody titer: Type III	C.F. antibody titer: Type I	C.F. antibody titer: Type II	C.F. antibody titer: Type III
44	4	4	I	1	0	0	0	0	0	0
		6	neg.	12	100+	10	0	32	0	0
3	5	10	I	7	32	0	0	0	0	0
		15	I	26	100	0	0	0	0	0
				47	100+	100+	0	32	0	0
30	5	12	I	8	100+	10	0	0	0	0
		14	I	30	100+	10	0	32	0	0
				51	100+	0	0	32	0	0
80	5	7–8	I	5	40	40	0	0	0	0
				24	160+	30	0	8	0	0
				39	160+	0	0	32	0	0
112	6	7–9	I	5	100+	32	0	0	0	0
				40	100+	0	0	8	0	0
				53	100+	0	0	8	0	0
7	9	7	I	4	10	10	0	0	0	0
				19	100+	0	0	4	0	0
				40	100+	0	0	8	8	0
187	32	8	I	4	160	160+	160+	0	0	0
				35	160+	80	160+	32	0	32

3. Type II virus was isolated from 15 patients, of whom 11 (73%) were paralytic and 4 (27%) were nonparalytic.

4. In no case was a Type III virus isolated.

5. When more than one specimen from a single patient yielded virus, the same type was recovered from each specimen.

6. Of the 40 paralytic cases, 26 (65%) yielded Type I virus, 11 (27%) Type II virus, and 3 (9%) no virus.

7. Of the 30 nonparalytic cases, 20 (67%) yielded Type I virus, 4 (13%) Type II virus, and 6 (20%) no virus.

Serological findings. 1. *Patients from whom Type I virus was isolated.* Serological studies were made on specimens from 18 patients with paralytic and from 14 with nonparalytic poliomyelitis in this category (Tables 4 and 5, respectively). The individuals reacted similarly, irrespective of the form of disease, and will therefore be dealt with together.

TABLE 5. SEROLOGICAL FINDINGS ON 14 PATIENTS WITH NONPARALYTIC POLIOMYELITIS FROM WHOM TYPE I VIRUS WAS ISOLATED

				Serological findings						
		Stool isolations			*Neutralizing antibody titer*			*C.F. antibody titer*		
Number of patient	*Age in years*	*Days after onset*	*Type of virus*	*Days after onset*	*Type I*	*Type II*	*Type III*	*Type I*	*Type II*	*Type III*
78	0.8	2	I	1	100	32	0	0	0	0
		3	I	19	100+	0	0	32	0	0
		6	I	42	100+	0	0	32	0	0
17	1.5	5	neg.	5	100+	0	0	4	0	32
		7	I	25	100+	0	0	32	0	32
		8	neg.							
50	2	4–5	I	4	0	0	0	0	0	8
		7	I	18	160+	40	0	0	0	8
				32	160+	10	0	16	0	8
54	2	7	neg.	7	100+	0	0	0	0	0
		8	I	41	100+	0	0	32	0	0
		9	I							
12	3	8	I	6	100+	0	0	0	0	0
		9	I	20	100+	0	0	32	0	0
86	3	18–21	I	25	100+	10	0	0	0	0
				64	100+	100+	0	32	0	0
113	3	2	I	1	32	0	0			
		4	neg.	22	100+	10	0			
		6	I							
48	7	4	I	4	10	10	0	0	0	0
		5–9	I	22	10	10	0	0	0	
				38	0	0	0			
				43				8	0	0
22	10	3	I	5	40	10	0	0	0	0
		7	I	23	160	0	0	8	0	0
				44	160+	0	0	16	0	0
40	10	8	I	7	100+	10	100+	0	0	8
		10	I	27	100+	0	100+	8	0	8
41	14	3	I	1	32	32	32	32	0	32
		6	I	24	100+	100+	100+	32	0	32

Number of patient	Age in years	Stool isolations: Days after onset	Stool isolations: Type of virus	Serological findings: Days after onset	Neutralizing antibody titer: Type I	Neutralizing antibody titer: Type II	Neutralizing antibody titer: Type III	C.F. antibody titer: Type I	C.F. antibody titer: Type II	C.F. antibody titer: Type III
46	14	8–9	I	4	40	40	10	0	0	0
				15	160+	40	10	0	0	0
52	15	12–14	I	12	100+	0	0	0	0	0
		15	I	28	100+	0	0	32	0	0
				54	100+	0	0	16	0	0
102	15	10	I	2	0	0	0	0	0	0
				46	160+	32	0	0[1]	0	0

[1] Greater than 4 when concentrated antigen was used.

Nineteen (59%) already had homologous neutralizing antibody in high titer (100 or more) in the first serum specimen. In subsequent samples these titers remained high. As end-points were not attained in the titrations, nothing can be said about the time when peak antibody titers were reached. Twelve others (38%) showed a rise in Type I antibody between their first and second serum samples. One patient (No. 48) showed a low titer in the initial bleedings with no subsequent rise.

Changes in Type II neutralizing antibody levels occurred in many of the patients who were infected with Type I virus. Twelve showed increases in Type II titer between the first and later sera. Twelve others had transitory low titer Type II antibodies detectable in the early sera only. Type III neutralizing antibody was present only in a few individuals and in only one (No. 41) was an increase in titer demonstrated.

With three exceptions (Nos. 41, 17, and 104) homologous C-F antibodies could not be detected in the first serum, but they appeared in the later specimens of all but four patients (88% positive response). When an antigen consisting of a tenfold concentrated TC fluid was used, a rise in titer from less than two to greater than four could be detected in two (Nos. 10, 102) of the three patients who were negative when tested with unconcentrated antigen.

Only one (No. 7) of the patients from whom Type I was isolated showed any Type II C-F antibody. In this one case it first appeared in the third serum. Nine (29%) had Type III C-F antibodies but during the period

TABLE 6. SEROLOGICAL FINDINGS ON 9 PATIENTS WITH POLIOMYELITIS FROM WHOM TYPE II VIRUS WAS ISOLATED

Number of patient	*Age in years*	*Stool isolations*		*Serological findings*						
					Neutralizing antibody titer			*C.F. antibody titer*		
		Days after onset	*Type of virus*	*Days after onset*	*Type I*	*Type II*	*Type III*	*Type I*	*Type II*	*Type III*
				Paralytic cases						
47	1.7	7–8	II	5	0	100+	0	4	0	4
				31	0	100+	0	0	16	0
11	4	8–10	II	6	32	100+	0	0	0	0
		11	II	28	0	100+	0	0	8	4
				49	0	100+	0	0	32	0
93	4	5	neg.	3	0	10	0	0	0	0
		6	II	33	0	100+	0	0	32	0
		8	II	44	0	100+	0	0	32	0
91	7	12	II	3	0	0	0	0	0	0
				23	0	100	0	0	8	0
				44	0	100+	0	0	32	0
97	7	11	II	7	40	40	0	4	0	0
		13	II	31	160+	160+	10	8	0	0
				59	160+	160+	10	16	4	0
117	28	11	II	5	100+	100+	0	0	0	0
				27	100+	100+	0	0	0	0
				54	100+	100+	0	0	0	0
				Nonparalytic cases						
137	1.8	4–6	II	2	100+	32	0	0	0	0
		8	II	28	100+	100+	0	0	0	0
18	12		II	0	100+	100+	0	16	8	8
			II	23	100+	100+	0	8	8	4
20	14	10	II	8	0	100+	0	0	0	0
		12	II	46	0	100+	0	0	0[1]	0
		14	neg.							

[1] Greater than 4 when concentrated antigen was used.

of study, the Type III titer increased in only two of these patients and it fell in two others.

2. *Patients from whom Type II virus was isolated* (Table 6). Nine of the patients studied were in this group. Five had homologous neutralizing antibody in high titer at the time the first serum was collected and maintained a high level for the period of study. The other four developed Type II antibodies in high titer by the time the second serum was collected.

One patient (No. 11) had transitory Type I antibodies. One (No. 18) had Types I and II neutralizing antibodies and C-F antibodies against all three types present in the first serum sample; of the C-F antibodies, only the Type II did not fall in titer in the second specimen. Another (No. 97) manifested simultaneous increases in antibodies to all three types of virus. It is noteworthy that No. 97 was the only patient in this group to show an increase in Type I C-F titer and that this increase was greater than that in Type II antibody. This raises a question as to whether Type I virus may also have been involved in this disease, for both viral types were prevalent in Lock Haven.

Four other patients showed increases in homologous C-F titer, and, when concentrated antigen was used, antibodies could be shown in the convalescent but not the acute phase serum of another (No. 20).

3. *Patients with clinical symptoms suggestive of poliomyelitis but from whom no virus was isolated* (Table 7). Nine patients in this group were studied, two paralytic and seven nonparalytic. Several of these patients showed serological responses to poliomyelitis virus and in certain cases the results give an indication of the type of virus that was involved. In three cases there was a rise in Type I C-F antibody and of these the two that were tested also had Type I neutralizing antibody present in high titer in all sera. They did not possess neutralizing antibody against either of the other two types.

Another patient (No. 99) had Type II C-F antibody in the convalescent but not in the acute serum. This individual had Type II neutralizing antibody in high titer in all three specimens and an increasing level of Type I neutralizing antibodies.

One patient (No. 66) had no detectable antibodies of either sort against any type of poliomyelitis virus.

4. *Integration of results from all categories of Lock Haven patients.* All persons studied had neutralizing antibodies against the type of virus isolated from their stools. In 24 of the 41 from whom virus was isolated (59%) this antibody was already present in high titer at the time of the

TABLE 7. SEROLOGICAL FINDINGS ON 9 PATIENTS WITH CLINICAL SYMPTOMS SUGGESTIVE OF POLIOMYELITIS FROM WHOM VIRUS ISOLATIONS WERE NEGATIVE* OR NOT TRIED

				Serological findings						
		Stool isolations			*Neutralizing antibody titer*			*C.F. antibody titer*		
Number of patient	*Age in years*	*Days after onset*	*Type of virus*	*Days after onset*	*Type I*	*Type II*	*Type III*	*Type I*	*Type II*	*Type III*
				Paralytic cases						
16	12	14	neg.	11	0	100+	0	0	0	32
				53	0	100+	0	0	0	32
14	18	12	neg.	4	100+	100+	100+	0	32	0
		14	neg.	20	100+	100+	100+	0	32	0
		20	neg.	67	100+	100+	100+	0	32	0
				Nonparalytic cases						
17	1.7	5	neg.	5	100+	0	0	4	0	32
		7	neg.							
		8	neg.	25	100+	0	0	32	0	32
66	6	3	neg.	3	0	0	0	0	0	0
				23	0	0	0	0	0	0
				44	0	0	0	0	0	0
51	7	17	neg.	17	0	0	32	0	0	0
		18	neg.	35	0	0	32	0	0	0
		19	neg.							
1	12			7				0	0	0
				51				32	0	0
8	12	6	neg.	3	100+	0	0	0	0	0
				21	100+	0	0	4	0	0
				50	100+	0	0	16	0	0
99	13	8	neg.	3	32	100	0	0	0	0
				20	100	100	0	0	32	0
				51	100+	100	0	0	32	0
188	20			12				0	0	4
				28				0	0	0

* By both suspended cell culture and outgrowth cell cultures.

first serum specimen. Increases in heterologous-type neutralizing antibody were found in 13 of 41 cases (32%).

Most persons (90% when the results with concentrated antigen are included) showed an increase in C-F antibodies during the period of study against the type of virus isolated from their stools. Heterologous C-F antibody increases occurred less frequently; 10% of the cases showed an increase in antibody against one or the other heterologous type.

The results, recorded in Tables 4, 5, 6, and 7, of the Type I neutralization and C-F tests on all the sera with which both tests were made (from 47 Lock Haven patients) are brought together in Table 8. All but one of

TABLE 8. NEUTRALIZATION AND COMPLEMENT FIXATION RESPONSES IN LOCK HAVEN POLIOMYELITIS PATIENTS

Neutralization titers during course of illness	*C-F titers*						
	Negative	*Fall in titer*	*Rise in titer* 2x	4x	8x	16x	*High titer in all samples*
	Number of patients in each category						
Negative	6	1	0	0	0	0	0
Fall in titer	1	0	0	0	0	0	0
Rise in titer	2	0	2	2	2	7	1
High titer present in all samples	3	1	1	2	6	10	0

the increases in Type I C-F were accompanied either by a concurrent increase in neutralization titer or a continuing high level of neutralizing antibodies. Concerning Type II serologic tests, more convalescent sera possessed neutralizing antibody than C-F antibody but eight of nine patients with a C-F antibody increase also showed an increase in, or continuing high level of, neutralizing antibody. There were too few patients with an increase in either Type III C-F or neutralizing antibody to bear analysis but the few that were positive for one kind of antibody showed little correlation with those positive for the other.

DISCUSSION

This report is concerned with the application and development of tissue culture methods for use in poliomyelitis laboratories concerned with diagnostic and epidemiological work. Three aspects have been investigated:

1. *Techniques for isolating virus.* Comparative tests of suspended cell cultures of monkey kidney tissue and of cultures of outgrowth cells indicated that the latter were more sensitive for the isolation of poliomyelitis virus. When large amounts of virus were present, virus could be isolated by either method, but for small amounts, the outgrowth cell cultures proved superior.

By the use of outgrowth cell cultures, virus could be isolated and typed in five to seven days if a moderate amount of virus was present and the specimen was not highly toxic.* A few days longer were required when suspended cell cultures were used for the primary isolation of virus. Suspended cell cultures have the advantage of circumventing the cytotoxic action of certain stools. However, cytotoxicity of stools is a far more serious problem with outgrowths from monkey testicular tissue than with those from monkey kidney tissue.

2. *Isolation and typing of poliomyelitis viruses from clinical specimens obtained in areas of low and of high incidence of the disease.* All three viral types were found in about equal proportions in New Haven patients in 1952, a year when the disease in the area was mild and the prevalence low. Type I and Type II viruses were both isolated during a severe outbreak in Lock Haven, Pennsylvania, during the same year. However, in the latter epidemic Type I strains were isolated at three times the frequency of Type II strains. There was no correlation in either area of strains of one type with a clinical form of the disease. Type II infections were as severe as those due to Type I, and the ratio of nonparalytic to paralytic infections among hospitalized patients was no greater for Type II than for Type I virus.

It has been shown above that outgrowth cell cultures are more sensitive in detecting small amounts of virus than are suspended cell cultures. Since all but three isolations from the clinical specimens were made by the suspended cell culture technique, some specimens reported here as negative undoubtedly contained virus. It is noteworthy that 58 of 70 patients (83%) from an epidemic area (Lock Haven) yielded virus, in contrast to 18 of 34 patients (53%) in an endemic area (New Haven). Perhaps this may be a

* A more rapid method can be used if adequate supplies of immune sera are available. The stool suspension in 0.25 ml. amounts is distributed in three tubes, the first containing 0.25 ml. of 1:5 Type I immune serum, the second, Type II, and the third, Type III serum. After an incubation period of one hour at room temperature, 0.2 ml. of each mixture is inoculated into each of two outgrowth cell cultures. The virus type can be identified as soon as it is isolated, for degeneration will be observed in the four tubes containing the heterotypic sera, but not in the two tubes containing the homotypic serum. By this technique, which has also been used at the Children's Hospital in Boston, virus can sometimes be isolated and typed within 48 hours.

reflection of a larger output of virus by patients infected by epidemic strains of poliomyelitis virus, or of a greater cytopathogenicity possessed by epidemic strains.

3. *Serological findings in patients.* It is now known that neutralizing antibodies to poliomyelitis virus develop early in the disease, so that it is not unexpected to find them present at high titer at the time the patient is brought into the hospital. The fact that the response may be broader than to the type causing the infection also makes it difficult to depend on neutralizing antibody measurements alone for diagnostic purposes. The discovery by Sabin[13] that transitory Type II neutralizing antibodies may appear in the face of a Type I infection has been confirmed. In one instance the reverse was found. Type I antibodies were present only in the first of three serum samples in a patient who excreted Type II virus and developed Type II antibodies in high titer. During convalescence some patients developed antibodies not only to the type isolated from the stools, but to other types as well. Whether these heterotypic antibodies persist for years like the homotypic ones is not known. Further study is needed before the rôle of these phenomena in the immunology of the disease may be understood.

There can be little doubt as to the identity of the infecting virus in those persons from whom virus was isolated, and in whose sera only homotypic C-F antibody increased in titer while homotypic neutralizing antibody was present in high or increasing levels. By these criteria 22 of the 40 persons on whom complete data were obtained had Type I and four had Type II infections.

When virus isolations were negative or not tried, the serological results alone were sometimes indicative of the type of infection. Of seven individuals in this category, two fulfill the above serological criteria for Type I infection and one for Type II. One possessed no antibodies against any type and probably did not have poliomyelitis.

One patient (No. 97), from whom Type II virus was isolated, showed increases in both C-F and neutralizing antibodies against both Types I and II. This may have been a heterotypic antibody response, but since both types were active in the community at the time of the study, there is a possibility that this was the result of a dual infection. Another individual (No. 7), from whom Type I virus was isolated, developed C-F antibodies against both Types I and II but possessed only transitory neutralizing antibodies against Type II. This is the kind of heterotypic response which has been found frequently by others.[2, 13, 15]

The C-F responses found in this study appeared later in the course of the disease and were more frequently monotypic than the responses described

by other workers.[2, 15] Possible reasons for these differences have been discussed in a previous paper.[1]

SUMMARY

Primary isolations of virus from human stools and throat swabs and from chimpanzee stools were made in suspended and in outgrowth cell cultures prepared from monkey kidney tissue. It was found that the outgrowth cell cultures were more sensitive in detecting small amounts of virus, and that the cytotoxicity of stools was not frequently a serious problem with kidney outgrowth cells. A period of three to five days was required for the virus to reach maximal titer in the primary cultures and a further three to four days was required for typing the virus by neutralization tests in a subsequent passage. The over-all period necessary for virus identification can be shortened if the primary isolations are made in the presence of type specific immune serum for each of the three virus types.

In New Haven, Connecticut, where the incidence of poliomyelitis was low in 1952, all three types of virus were isolated. From stool specimens Type I virus was isolated from five patients, Type II from eight, and Type III from five. Throat swabs yielded virus less often than stools, but even the seven strains isolated from throat swabs were distributed among the three types of virus. In Lock Haven, Pennsylvania, where a severe outbreak of poliomyelitis occurred in 1952, only Types I and II were isolated. Type I virus was isolated from the stools of 46 patients and Type II from 15.

The results of neutralizing antibody tests against the three types of poliomyelitis virus confirm the recent observations made in several laboratories that such antibodies develop rapidly and are usually present at the time the patient is brought into the hospital. In the present study, of 41 patients from whom poliomyelitis virus was isolated, only 15 showed higher concentrations of homotypic antibody in the convalescent serum specimens than in acute phase specimens, whereas in 25 patients the homotypic antibody was present in high titer throughout the period of observation. Sabin's findings on the appearance of transitory heterotypic neutralizing antibodies during the acute phase of the disease have been confirmed. Other patients developed heterotypic neutralizing antibodies in the convalescent phase.

Determinations of complement-fixing antibodies in the same sera made with minimal amounts of antigen revealed antibody responses homotypic with the virus isolated from the stools in a high percentage of patients (90%) and heterotypic in comparatively few (10%). These antibodies did not generally become detectable until more than one week after the onset of the disease and often increased after the fourth week.

No correlation was found between the type of infecting virus, the severity of the disease, and the immunological response. Each of the three bulbar cases from New Haven yielded a different type of virus. Of the 46 Lock Haven patients from whom Type I virus was isolated, 57% were paralytic; of the 15 from whom Type II was isolated, 73% were paralytic. The serological responses were similar in the paralytic and nonparalytic cases and in the Type I and Type II infections.

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THE SPONTANEOUS PROTEOLYTIC ACTIVITY OF DOG BLOOD*

Although there is little doubt that more than one proteolytic enzyme occurs in plasma, plasmin (fibrinolysin) is the only such enzyme which has been definitely identified.

Astrup and Permin,[1,2] on the basis of differences in susceptibility to activation by streptokinase and fibrinokinase, have suggested that there are two precursors of fibrinolytic enzymes in human blood. They have designated the enzymes activated by streptokinase and fibrinokinase, respectively, as s-plasminogen and f-plasminogen, the latter being responsible for the spontaneous proteolytic activity of blood.

The suggestion of Astrup and Permin that the enzyme producing the spontaneous proteolytic activity of serum is not identical with plasmin, which requires external activators such as chloroform or bacterial kinases, was investigated in the dog, and the results are reported in this paper.

MATERIALS AND METHODS

The euglobulin fraction of the plasma proteins was precipitated from dog plasma or serum by the method of Milstone.[3] Proteolytic activity was assayed under the conditions described by Downie and Cliffton.[4] The tyrosine-like chromogenic materials in trichloroacetic acid filtrates after incubation was estimated by (a) the development of color with Folin-Ciocalteu reagent,[5] or (b) the absorption at 280 mμ measured in the Beckman DU Spectrophotometer.[6]

Fibrinolytic activity. A clot was formed by the addition of commercial thrombin to fibrinogen (Armour). Total dissolution of the clot was taken as the end point.

Activation. Staphylokinase, the activator employed, was precipitated with alcohol from the supernatant culture medium of a strain of Staphylococcus known to have produced staphylokinase. The precipitate was dried with absolute alcohol and ether, further dried in a vacuum desiccator, and ground to a fine powder.

Heparin. Commercial Liqueamin, 0.25 ml. per experiment, was used. This has since been found to be greatly in excess of the amount required to produce the inhibitory effects observed.

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RESULTS

EVIDENCE THAT SPONTANEOUS ENZYME IS NOT PLASMIN

1. *Differential action of inhibitors*

(a) Effect of heparin. The addition of heparin to whole blood or serum resulted in almost complete inhibition of the spontaneous activity of the euglobulin fraction (Table 1). On the other hand, plasmin activity, measured after staphylokinase activation (total activity minus spontaneous), was not affected by the addition of heparin ($P > .05$).

TABLE 1. EFFECT UPON PROTEOLYTIC ACTIVITY* OF THE ADDITION OF HEPARIN AND OXALATE TO DOG BLOOD OR SERUM

	n	*Spontaneous* (a)	*Total*† (b)	*Plasmin* (b) — (a)
Serum	9	.278 ± .005	1.10 ± .02	.822 ± .020
Blood or serum plus heparin‡	14	.049 ± .005	.841 ± .040	.792 ± .040
Blood plus oxalate	10	.264 ± .006	.818 ± .030	.554 ± .031

* Absorption of trichloroacetic acid filtrate at 280 mμ.
† Staphylokinase activated.
‡ 0.25 ml. liquaemin.

(b) Effect of oxalate. The addition of oxalate to blood had no effect upon the spontaneous proteolytic activity ($P > .05$) but plasmin activity was significantly decreased ($P < .01$) (Table 1).

(c) Effect of the addition of heparin to the euglobulin fraction of serum. Heparin, when added after the precipitation of the euglobulin fraction, exhibited a much smaller, although significant, inhibitory effect upon the spontaneous proteolytic activity ($P < .01$) (Table 2). Plasmin activity was unaffected ($P > .05$).

(d) Serum inhibitor. Serum, diluted 1:20 with saline, was found to inhibit the spontaneous proteolytic activity of the euglobulin fraction ($P < .01$), whereas plasmin activity was not decreased ($P > .05$) (Table 2).

(e) Heparin plus serum inhibitor. The last series of experiments in Table II shows that the addition of both heparin and serum inhibitor to the euglobulin fraction of serum produced inhibition of the spontaneous activity

($P < .01$) which appeared to be a summation of their individual effects. Plasmin activity was not decreased.

2. *Comparison of action against casein and fibrin clots.* By appropriate dilution, solutions of euglobulin were prepared which, when activated with staphylokinase, digested casein to the same extent as undiluted solutions without activation. When tested against fibrin clots, the activated solutions produced complete clot lysis within five minutes, whereas the unactivated solutions failed to lyse the clots within 24 hours.

TABLE 2. EFFECT UPON PROTEOLYTIC ACTIVITY* OF ADDITION OF HEPARIN AND SERUM INHIBITOR TO THE EUGLOBULIN FRACTION OF DOG SERUM

Addition	n	*Spontaneous* (a)	*Total*† (b)	*Plasmin* (b) — (a)
None	4	.419 ± .030	1.11 ± .07	.691 ± .057
Heparin‡	4	.312 ± .023	1.08 ± .06	.768 ± .012
Serum inhibitor§	4	.347 ± .067	1.11 ± .08	.763 ± .032
Heparin plus serum inhibitor	4	.217 ± .046	1.13 ± .07	.913 ± .025

* Absorption of trichloroacetic acid filtrate at 280 mμ.
† Staphylokinase activated.
‡ 0.25 ml. liquaemin.
§ There is no significant difference between the averages obtained for uninhibited and serum-inhibited euglobulin because of the variation between animals. In each paired experiment, however, an inhibition was observed. For the series, the effect of serum inhibitor was highly significant ($P < .01$).

3. *Site of action in casein molecule.* In two experiments, successive 2 ml. portions of fresh euglobulin solution were added at intervals to a flask containing 25 ml. of the usual casein medium until no further proteolysis occurred. The extent of proteolysis was estimated by the periodic removal of 3 ml. aliquots to which 3 ml. of 10% trichloroacetic acid was added to precipitate undigested casein. After filtration, the absorption at 280 mμ was measured. When proteolysis ceased, as indicated by the maintenance of a constant value for two successive days, 0.4 ml. of 1% staphylokinase solution was added in addition to fresh euglobulin solution. The resumption of hydrolysis is evident (Table 3). In the second experiment, as an additional control, after exhaustive digestion by unactivated enzyme, the incubation and control tubes were each divided into two equal portions. Fresh euglobulin solution (boiled in the case of the control tubes) was added to one, and

the same enzyme solution plus 0.4 ml. of 1% staphylokinase solution was added to the other. As may be seen in Table 3, the tube containing staphylokinase showed a renewal of proteolytic activity, whereas the tube without added activator showed no further hydrolysis of casein during this 24-hour period. The final column in Table 3 gives the optical densities of the trichloroacetic acid filtrates corrected to the previous volume to eliminate the dilution effect caused by the addition of fresh enzyme solution.

TABLE 3. FURTHER PROTEOLYSIS OF CASEIN BY STAPHYLOKINASE-ACTIVATED EUGLOBULIN AFTER EXHAUSTIVE PROTEOLYSIS BY UNACTIVATED EUGLOBULIN

Experiment	*Time*	*Addition*	*Optical density of TCA filtrate at 280 mμ**
1†	0 hours	Euglobulin	....
	15 "	"	.550
	48 "	"	.799
	70 "	"	.873
	96 "	" + staph.	.799
	132 "	.....	1.500
2	0 hours	Euglobulin	....
	15 "	"	.658
	24 "	"	.718
	36 "	"	.959
	‡72 "	(a) "	.975
		(b) " + staph.	
	96 "	(a)	.960
		(b)	1.644

* Corrected for volume changes so that successive samples may be compared directly.
† In experiment 1, control tube contained buffer. In experiment 2, boiled enzyme solution.
‡ Solution divided into two equal portions.

DISCUSSION

In view of the finding that the spontaneous and activated proteolytic enzymes of dog blood differ with respect to the effects of inhibitors, their activity against different substrates, and the bonds in casein which are attacked, it appears that the spontaneous activity represents the action of an enzyme which is distinct from plasmin.

The differentiation of the proteolytic enzymes of serum has proven difficult in that no specific simple substrates for plasmin were known.

A recent report[7] of the finding of such substrates may help to clarify the situation, although the proteolytic activity of serum is so weak that concentration of the spontaneous activity may be necessary before definitive results based on substrate specificity can be obtained.

Astrup and Permin[2] have suggested the designations s-plasminogen and f-plasminogen for the enzymes in human blood which are activated by streptokinase and fibrinokinase, respectively. In view of the fact that a variety of activators, varying from species to species, have been described, it would appear less confusing at this time to designate the enzyme responsible for the spontaneous proteolytic activity of serum as Plasmin B, retaining the established name plasmin (fibrinolysin) for the enzyme activated by the kinases and chloroform.[3]

SUMMARY

Evidence is advanced from inhibition studies, differential action against substrates, and a difference in the bonds in casein which are attacked that the spontaneous proteolytic enzyme of dog serum is not plasmin.

The designation Plasmin B is tentatively suggested for the enzyme responsible for the spontaneous proteolytic activity.

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THE SIGNIFICANCE OF FEVER IN INFECTIONS*

INTRODUCTION

Although body temperature had been measured sporadically as a sort of novelty for a number of years, it was not until 1868 when the German physician, Wunderlich, published the results of his monumental researches on clinical thermometry that the tremendous usefulness of the thermometer as an instrument of physical diagnosis was widely recognized. Wunderlich's book, the compilation of sixteen years of observations on body temperature in disease, was translated into English in 1871;[40] the preface to this edition contains the following statement:

> The more my observations were multiplied the more firmly rooted did my conviction become of the unparalleled value of this method of investigation, as giving an accurate and reliable insight into the condition of the sick.

The years have justified Dr. Wunderlich's conviction. The standard practice of recording patients' temperatures has become so taken for granted that many older observations which enabled the physician to draw definite, albeit limited, conclusions from simple inspection of the graphic record of a patient's febrile course are often neglected or forgotten. Much of the deemphasis of fever (as well as other physical signs) is a matter of "one sees only what one looks for." However, the "modern" tendency to substitute laboratory tests for history and physical examination and the temptation of the "easy out" offered by the availability of antibiotics are probably equally responsible. While few would deny categorically the suggestion that fever can be a valuable indicator of the correct diagnosis, of the severity or duration of an illness, of therapeutic response, of relapse or complication, or, indeed, of the presence or absence of an organic basis for complaints, it would seem useful to review the significance of fever in the present-day management of acute infections.

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THE MECHANISM OF FEVER WITH INFECTIONS

Although the physiological mechanisms responsible for maintaining the balance between heat production and heat loss have been subjected to numerous experimental studies, surprisingly little attention has been paid to the ways in which disease disturbs this equilibrium. Many bacteria, particularly Gram negative organisms,[7] and a few viruses[16, 18, 37] elaborate "pyrogens" or toxins which provoke an elevation of body temperature when injected into man or animals; the familiar use of typhoid vaccine for fever therapy is an example. However, numerous pathogenic microörganisms possess no detectable pyrogen, and fever also occurs with diseases in which there is no invasion of the host by an infectious agent. These include: neoplastic diseases (lymphoma or hypernephroma); hemolytic crises; hypersensitively states (serum sickness, drug fevers and, perhaps, the "collagen diseases"); vascular occlusions (pulmonary or myocardial infarctions); and metabolic abnormalities (gout and porphyria). A common denominator in these afflictions is tissue injury and the hypothesis most often advanced states that fever results from the action upon cerebral regulatory centers of substances released from damaged cells. Without going into detail, it may be stated that convincing experimental proof of this hypothesis is exceedingly difficult to come by although some evidence is at hand.[5, 8, 9, 31, 32] However, our lack of understanding of the basic mechanism of a symptom or sign as common as fever is by no means unique. We are equally ignorant as to the mechanism of leukocytosis and leukopenia; even the factors responsible for the localization of the skin manifestations of infections are mysterious. For instance, the rash accompanying murine typhus begins on the trunk and spreads distally, while the rash of Rocky Mountain spotted fever, another rickettsial disease, starts on the extremities and spreads proximally. We do not know why rose spots in typhoid appear on the abdomen or why the circumoral region is spared in scarlet fever. When it comes to such bizarre examples as the erythema of pretibial or Ft. Bragg fever, which is often limited to the shins, we are totally lost.

Before turning to a discussion of some factors to be considered in evaluating the clinical significance of fever in the management of acute infections, it is pertinent to point out that the febrile course of many diseases, infectious or non-infectious, is so irregular that practically no definite conclusions are possible. Furthermore, none of the points to be discussed in regard to fever in specific diseases is invariable enough to warrant interpretation of elevated body temperature as a physical sign with any less caution than is used in evaluating other physical findings such as those elicited by auscultation of the heart and lungs. Lastly, conclusions after

inspection of a patient's fever chart must often be modified after consideration of all information that is available from history, physical examination, and laboratory tests.

CHILLS AND RIGORS

The fever of infection may be insidious in onset or begin suddenly with a shaking chill. It is important to distinguish chilliness or chilly sensations which are common with all fevers from true rigors. Shaking chills are common in some infections, unusual in others. For example, when jaundice and fever occur together, it is well to remember that chills are rare in viral hepatitis but are common in cholangitis secondary to stones in the biliary system. Although chills are mentioned in some descriptions of infectious hepatitis, it is probable that the distinction between chilliness and chills was not made. If the patient's chief complaint is pleuritic pain, the rarity of rigors with pulmonary infarction as compared to their frequency in bacterial pneumonias may be helpful in diagnosis. The number of chills is sometimes of significance. Repeated chills over a period of several days suggest bacteremia or in an endemic area, malaria. The common practice of taking aspirin or some other antipyretic at home, however, can result in repeated sweats and rigors which will mislead the physician who takes the history unless the patient is asked specifically about such medication. It is uncommon for pneumococcal pneumonia to cause more than a single chill;[12] a history of repeated chills in a patient with pneumonia who has taken no antipyretic strongly suggests an etiologic organism other than the pneumococcus. Not all chills are due to infections; Hodgkin's disease, hypernephroma, and hemolytic crises are examples of other diseases which may be accompanied by shaking chills.

In bacterial infections, chills are often the signal of transient invasion of the blood stream by the infecting organism. The train of events is similar to that following the intravenous injection of typhoid vaccine in fever therapy where the rise in temperature, with its unpleasant accompanying symptoms, does not begin until more than an hour after introduction of the pyrogen into the blood stream. A lag period of ninety minutes to two hours is typical[7] and is of considerable importance not only from a theoretical viewpoint but also in the very practical matter of obtaining blood cultures. Bacteria which enter the blood stream are ordinarily removed with great rapidity by the cells of the reticulo-endothelial system and, as has been demonstrated on numerous occasions, blood cultures obtained at the time of a chill may show no growth, the offending organisms having long since been phagocytosed.[10] For this reason, in a patient with repeated chills or

spiking fever without chills, an effort should be made to obtain diagnostic blood cultures during the hour or two before the expected rise in temperature. Figure 1 is a portion of the temperature chart of an elderly woman with chronic meningococcemia. She had had intermittent chills during the preceding three weeks and developed a typical rash consisting of erythematous macules and papules with hemorrhagic centers shortly before admission. Of ten blood cultures taken, as indicated by the arrows, only one was positive, and, in this instance, the bacteria grew in blood obtained during the downswing of a temperature spike. This apparent exception to the rule

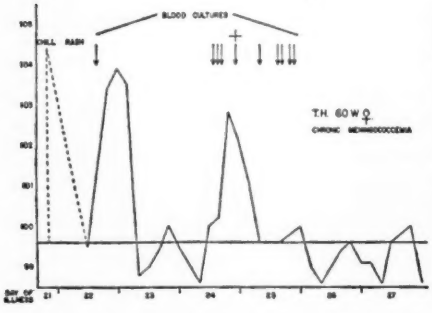

FIG. 1. Temperature record of elderly woman with chronic meningococcemia. Of ten blood cultures taken (indicated by arrows) the only one showing growth was obtained as fever was subsiding after a sudden rise.

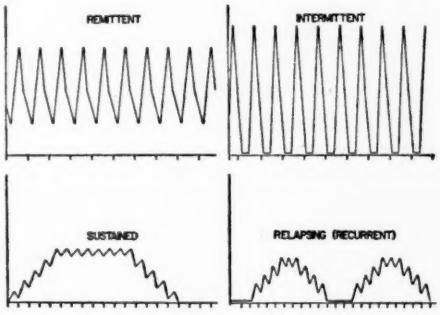

FIG. 2. Diagrammatic representation of some of the common patterns of fever.

just stated is cited to emphasize two points: first, blood cultures obtained at this stage *can* be diagnostic and, while it is preferable and often helpful to anticipate the fever, multiple cultures during the day are certainly justified as a diagnostic procedure in febrile patients; second, in this specific infection, chronic meningococcemia, numerous cultures over a period of several days may be required to isolate the causative organism.

Lastly, the treatment of a chill deserves a word. Quite apart from the sudden rise in body temperature, headache, back-pain, nausea, and uncontrollable shivering unite to render the patient a miserable and pitiful sight as he burrows under the blankets with chattering teeth seeking in vain to warm himself. The rapid intravenous injection of 10 or 20 ml. of 10 per cent calcium gluconate will usually stop the rigor abruptly and relieve most of the patient's malaise although the subsequent rise in temperature is not appreciably affected.[5] The mechanism of this effect is not clear; the increase in heat-production occasioned by the rhythmic muscle contractions during

chilling, which may be as much as 600 per cent,[18] is apparently made up by the liver or by continuous muscle activity without the unpleasant jerking movements, or is compensated by further decrease in heat loss through intense peripheral vasoconstriction. At any rate, the alleviation of discomfort is often dramatic, and the patient's esteem for his physician usually rises despite the fact that the doctor often not only stopped the chill but also initiated it by administration of pyrogen-contaminated glucose or blood.

TYPES OF FEVER CURVES

Inspection of the graphic record of a patient's febrile course is a source of information useful to the clinician. Recurrence of fever may signal relapse or complications; in typhoid, abrupt defervescence is sometimes the first indicator of intestinal hemorrhage.

The shape of the curve may be enough to suggest a correct diagnosis or to eliminate some of the diseases under consideration. Figure 2 presents, in diagrammatic fashion, examples of some of the well-known types of fever:[4] *remittent,* in which there are wide variations during the day but the temperature never returns to normal; *intermittent* or *quotidian,* in which there are daily spikes with return to the baseline in the interval; *sustained,* in which there is fever throughout each day with variation of less than 2° F. during the 24 hours; and recurrent or *relapsing,* consisting of bouts of fever for a few days separated by periods of normal temperature. When remittent or intermittent fevers show wide daily swings, often with repeated chills, the term *hectic* or *septic* fever is used. Such a curve suggests pyogenic infection, often with localized suppuration. In this country, the presence of a recurrent or relapsing type of fever suggests malaria, rat-bite fever (due to *Spirillum minus* or *Streptobacillus moniliformis*), relapsing fever (caused by *Borrelia hermsi* or *parkeri* in some Western states), or chronic meningococcemia.[4] One special type of recurrent or relapsing fever, the "saddle-back" or "dromedary" curve, is usually described as typical of dengue; actually it is only occasionally seen.[34] A number of other virus diseases are characterized by a few days of fever followed by a period of normal temperature and then recurrence of fever. Poliomyelitis, particularly in children, consists of a minor illness followed in a day or two by the major illness. Colorado tick fever, a relatively common virus infection in Colorado, Idaho, Utah, and Wyoming, is characterized by two bouts of fever and malaise separated by a symptom-free interval of two days. Smallpox is often "camel-back" in onset and in yellow fever, the stages of active congestion and of stasis are usually separated by an afebrile period.

Sustained fever is typical of rickettsial infections; it is the rule in pneumococcal pneumonia, and the classic fever of untreated typhoid is sustained or mildly remittent.

Certain of the classic fevers deserve comment. The Pel-Ebstein type of recurrent fever of Hodgkin's disease is seen in fewer than 5 per cent of cases. Of undulant fever or brucellosis, Spink says:[35]

> Contrary to classic descriptions of the disease, the temperature curve does not usually exhibit a remittent or undulating type of fever . . . The usual course is that of an intermittent fever with diurnal variations from 98° up to 100° to 104° F. Occasionally a sustained fever may take place, as observed in typhoid fever.

A fever curve with two spikes during each 24 hours, the so-called "double quotidian" curve should suggest gonococcal endocarditis or kala-azar.[21] Endocarditis due to the gonococcus has become extremely rare but careful studies before penicillin was available confirmed the presence of such a febrile curve in about 50 per cent of cases.[19] Kala-azar is not likely to cause great concern in the differential diagnosis of infections in this country.

The normal diurnal gradient of body temperature is worth bearing in mind in examining fever charts. During the day, the body temperature increases from an oral level of about 97.5° on awaking in the morning to as high as 99.2° in the early evening. During the night, it falls gradually to its lowest level at 2-4 a.m. and rises slowly after this time. All the factors responsible for this gradient are not clear. Increasing activity during the day, and rest at night are usually stated to be responsible for these changes, the rise beginning at 2-4 a.m. being attributed to restlessness prior to awaking. Other factors are probably operative, however, since patients confined to bed continue to show this pattern of change and the extensive studies of Kleitman[26] showed that reversal of activity such as work on the "swing-shift" or as a night-watchman, contrary to popular belief, does not change this gradient. In febrile patients, daily variations in body temperature are usually greater than normal, but ordinarily the diurnal gradient remains the same; fever tends to be higher in the late evening and the spikes of a hectic fever usually come at 4-8 p.m. Night sweats, usually (but not invariably) an evidence of low-grade fever, tend to occur at the low point in the gradient, 2-4 a.m. This fact may be of aid in differentiating the night sweats of chronic infection from those of emotional disturbance which tend to occur sooner after retiring.

Reversal of the gradient occurs; patients may have shaking chills in the early morning or may spike high fever in the forenoon. In my own experience this has occurred most often in tuberculosis or in bacteremias due to

Salmonella organisms. Figure 3 shows a hectic fever in a patient with pulmonary tuberculosis and illustrates an interesting clinical finding which suggests that the diurnal gradient may involve factors more subtle than mere activity. In this patient, there was a high spike each morning for many days. He was given an antipyretic, amidopyrine, at four-hour intervals for several days. The drug was finally discontinued abruptly at 8 a.m. and as its influence wore off during the afternoon, fever returned and the tempera-

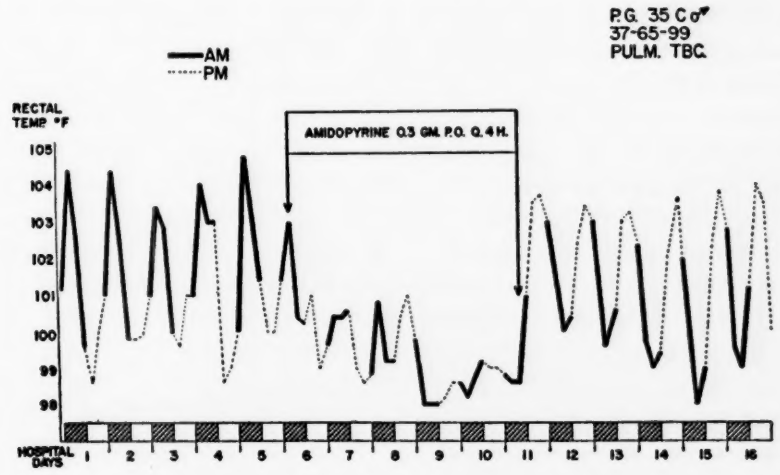

FIG. 3. Temperature record of a patient with advanced tuberculosis whose hectic fever showed daily morning rises, the reverse of the normal daily gradient of temperature changes. After a period of suppression of fever by amidopyrine, the hectic fever returned, but at this time conformed to the gradient by showing the usual pattern of an evening rise each day.

ture promptly spiked between 4 and 8 p.m. The hectic pattern was resumed and maintained in the pattern of a normal diurnal gradient. It was not possible to tamper with this patient's gradient in further studies as he was transferred to another hospital. While no sweeping conclusions can be drawn from this single chart, further attempts to alter the cycle of febrile or normal subjects by such a procedure may give interesting results.

THE HEIGHT OF FEVER

The degree of temperature elevation is sometimes of importance. Children, of course, tend to respond with higher fever to many infections than do adults. It is also true that elderly patients occasionally show surprisingly little temperature elevation in the presence of severe infection, particularly pneumonia.

Oral temperatures exceeding 105° F. in adults are relatively unusual and suggest certain diagnostic possibilities: malaria, infections due to Gram negative bacilli such as pyelonephritis, typhoid, or brucellosis, and tuberculosis should be considered. Relapsing fever is apt to be accompanied by extreme elevations of temperature.

Some severe infections, particularly those due to organisms which elaborate exotoxins, are accompanied by little fever. Botulism, really not an infection but an intoxication, is an afebrile disease. Diphtheria is rarely accompanied by a fever of more than 102° except in rapidly fatal cases. Fever in the course of tetanus is a grave prognostic sign.

Much attention has been paid to failure of the pulse to accelerate in proportion to rise in body temperature; relative bradycardia is sometimes cited as being pathognomonic of typhoid. Actually, a large number of viral infections are also accompanied by bradycardia, including psittacosis (which is easily confused with typhoid), infectious mononucleosis, mumps, dengue, and atypical pneumonia. Relative bradycardia in yellow fever is called Faget's sign.[30] It is worth remembering that many types of meningitis may present with relative bradycardia, presumably due to increased intracranial pressure. Obstructive jaundice, probably due to retained bile salts, is accompanied by bradycardia[28] and may rarely cause confusion, particularly if jaundice is recent in onset. In summary, bradycardia out of proportion to fever is so common that it can hardly be regarded as a useful sign of any specific infection.

THE DURATION OF FEVER

Some knowledge of the usual duration of fever accompanying specific infectious diseases is often of aid in diagnosis or prognosis. The fever of Weil's diseases is ordinarily of short duration, rarely extending more than 4-7 days; this point often enables one to eliminate this disease from consideration in the diagnosis of febrile jaundice. So-called "cat-scratch disease" is accompanied by relatively brief fever although impressive lymphadenitis often persists for weeks.[11] Persistence of fever in poliomyelitis is a poor prognostic sign. In patients with infectious hepatitis, fever of more than two weeks' duration almost invariably means that the diagnosis is incorrect or that a complication such as subacute hepatic necrosis is present.[25] So variable is the duration of fever in some diseases that it is difficult to draw conclusions. This is the case with atypical viral pneumonia and is one of the main reasons for disagreement about the effectiveness of aureomycin in this infection.[17, 20]

THE USEFULNESS OF FEVER TO THE PATIENT AND THE TREATMENT OF FEVER

The question of the rôle of fever in the body's defense against infection often arises. There is no doubt about the effectiveness of induced fever in diseases such as neurosyphilis, certain inflammations of the eye, and arthritis. In animal experiments elevation of temperature has been shown to be of importance in resistance to pneumococcal infections[15, 33] and to cryptococcosis.[27] On the other hand, increased nitrogen wastage with elevation of metabolism by fever (7 per cent per F. °), increased circulatory demands in patients with myocardial disease,[1] and, of course, hyperpyrexia itself are obviously undesirable. Lastly, the value of the temperature curve as an indicator of therapeutic response is worth considering before instituting antipyretic treatment.

There is little to indicate that lowering temperatures with aspirin impairs body defenses in human infections. Actually, the usual reason for the use of aspirin is to alleviate the uncomfortable symptoms which often accompany fever. Evidence that fever per se is responsible for malaise, listlessness, aches and pains in many infections is not convincing; relief of such manifestations by aspirin is hardly proof, as salicylates and related drugs are also analgesics. It is my own practice to employ codeine or demerol for relief of discomfort in most instances, thus preserving the fever as a guide to specific therapy. Some patients with high fever feel surprisingly well. Much of this, of course, depends upon the individual, but high fever in the absence of malaise is suggestive of tuberculosis; patients with this disease are often "pepped up" by fever and actually become more lively as the temperature rises each evening.

When hyperpyrexia or other considerations lead to a decision to lower the patient's fever, however, certain things are important. Mechanical measures are useful if immediate defervescence is desired. There is little hope that appreciable decrease in heat production will be accomplished in a short time; measures directed to increasing heat loss can be effective. By far the most efficient means of doing this is by evaporation of water from the skin. Tepid sponging of the extremities is the preferred procedure and the sponging should be vigorous. Alcohol evaporates rapidly but abstracts fewer calories than does water. Ice packs or cold sponges may intensify cutaneous vasoconstriction and actually cause a temperature rise. DuBois[14] has pointed out that the retention of only sixty calories will raise the temperature of the average adult 1° C. The ingestion of one liter of water at 0° C. (or the melting of this amount of ice on the skin surface) abstracts forty calories of heat, resulting in a drop of ⅔° C. in body temperature. The same amount of water vaporized from the skin will extract 585 calories.

Vigorous sponging stimulates cutaneous vasodilatation and the efficient evaporation of water.

If an antipyretic drug such as aspirin is used to make a patient comfortable it should be given in adequate dosage at four-hour intervals or a miserable cycle of repeated bouts of chilliness, sweating, etc., may ensue as fever comes and goes. The fevers accompanying some infections show remarkable differences in their susceptibility to antipyretics. The fever of typhoid is unusually susceptible to aspirin and doses of 0.3 to 0.6 g. sometimes result in such an abrupt fall in temperature that circulatory failure

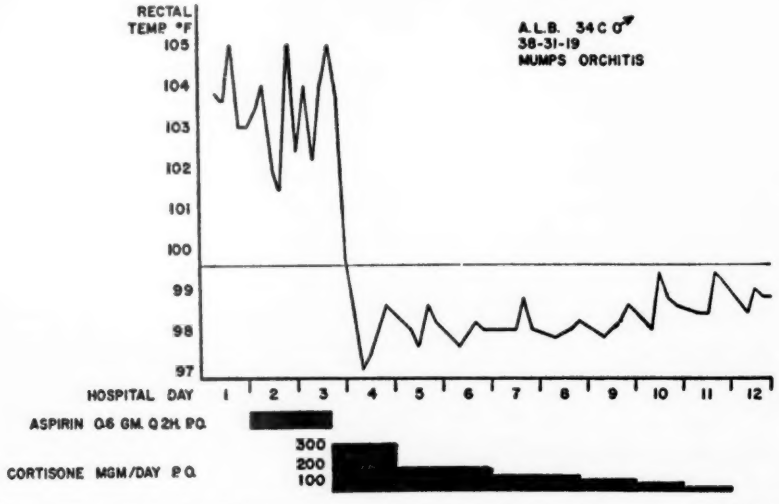

FIG. 4. The effect of cortisone upon the fever of a patient with mumps orchitis after failure of aspirin to induce defervescence.

supervenes. As a medical student, I saw a patient with typhoid given 0.6 g. of aspirin for hyperpyrexia of 107° F. In slightly more than an hour, the temperature had fallen to 96° F. and the patient was stuporous, cyanotic and hypotensive. Aspirin chills have been described as a characteristic of typhoid. Among fevers more resistant to antipyretics, two diseases have been impressive in my own experience, although documentation of this has not been found in published reports. The high fever of exanthem subitum or roseola infantum is said by many pediatricians to be resistant to salicylates in ordinary dosage. This clinical impression was of aid to me on the one occasion that I have diagnosed this illness in the pre-eruptive stage. The second type of fever which seems surprisingly resistant to aspirin accompanies mumps orchitis. These statements about roseola and orchitis

must be classified as clinical impressions at the moment, but we are in the process of using pencil and notebook, as Dr. Henry Beecher of Boston has advised,[2] in an effort to obtain records which will enable us to change these clinical impressions to documented clinical research.

Lastly, we would not be in style if we failed to mention cortisone at least once. This hormone has many influences upon infectious diseases and a discussion of the controversy over its beneficial or deleterious effects would be out of place here.[23] Most infectious fevers fall promptly when cortisone is administered and there is much sentiment and experiment to justify its use in conjunction with antibiotics in the treatment of severe infections such as typhoid.[38, 39] Figure 4 illustrates the effect of cortisone in a patient with mumps orchitis. Aspirin in a dosage of 7.2 g. daily was without appreciable effect upon the patient's fever. Striking defervescence followed a single oral dose of 300 mg. of cortisone. Incidentally, testicular pain and swelling were also greatly improved. In four additional cases, we have gained the idea, still an impression, that this hormone may prove to be extremely efficacious in the management of this troublesome complication.[22]

THE FEVER CURVE AS AN INDICATOR OF THERAPEUTIC RESPONSE

Subsidence of fever is, of course, a valuable indicator of the effectiveness of antibiotic treatment. Indeed, it has become fully as important for physicians to be aware of the expected patterns of response when antibiotics are administered as it is to recognize symptoms and signs in diagnosis. Otherwise, needless shifting to other drugs or undue concern about the correctness of diagnosis or undetected complications may ensue. There is a great tendency for many of us to regard the usually dramatic response of pneumococcal pneumonia to penicillin as a prototype of the response of infection to antibiotics. Figure 5 demonstrates fairly typical results of specific treatment in pneumococcal pneumonia, in typhoid fever where the effect of chloramphenicol is usually not apparent for 3-5 days, in meningococcemia, where a dramatic response to sulfadiazine occurs, and in meningococcal meningitis, in which very gradual subsidence of fever over several days follows sulfonamide, penicillin, or combined treatment. The differences in these curves illustrate the need for an acquaintance with the usual response to specific antimicrobial agents.

"F.U.O." AND INFECTION

I do not propose to enter into a discussion of the difficult diagnostic problem presented by so-called "fever of unknown origin" other than to point out that it still exists.[3] Dr. Kilbourne of Tulane has recently warned

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INDIVIDUALIZATION OF TEACHING ON THE PEDIATRIC CLERKSHIP AT YALE

In recent years medical educators have explored a number of educational techniques in an effort to improve current teaching practices. Introduction of clinical clerkships a number of years ago provided an opportunity for greater individualization of student experience during the third and fourth years. Instead of attending formal didactic lectures and demonstrations, the student was given the responsibility for a certain amount of patient care; thus, he might take the medical history, perform a physical examination, arrive at diagnostic considerations, perform indicated laboratory as well as other diagnostic and therapeutic procedures, and participate in the planning of therapy. The recent increase in the size of clerkship groups has tended, however, to compromise the benefits of this educational approach. Consequently, some alteration in the present conduct of clerkship teaching would appear indicated. We wish to report on two phases of the third-year pediatric clerkship program at the Yale University School of Medicine designed to meet some of the problems presented by large clerkship groups and to accomplish the purposes of medical education as we view them. These consist of the utilization of a tutorial system and the employment of personal interviews to discuss student performance.

THE TUTORIAL SYSTEM

The tutorial system has, of course, long been used in collegiate education but has not had widespread application in medical education. A trial of the tutorial system on the pediatric clerkship at Yale was suggested by Darrow, and the program described below has been in effect for two years. On the basis of personal observations and student comment, we believe that this educational technique has a definite place in clinical clerkship teaching.

An average of 12 students is assigned to the pediatric ward clerkship for a period of six weeks during the junior year; the system of rotation provides clinical experience on the pediatric medical ward, the pediatric infectious disease ward, in the newborn nurseries, the rooming-in nurseries, and the emergency room. A few home visits are made with the interne assigned

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to the nursery, and some of the students elect to spend one day with a pediatrician in private practice. The tutorial sessions are usually held for one hour twice a week with two students assigned to each tutor for the duration of the clerkship. Assignments as tutors are given to qualified research fellows as well as to more senior members of the Department. The opportunity to gain teaching experience and to keep in touch with patients being studied on the wards has been highly valued by these men who are beginning an academic career; the two hours a week allotted for the tutorial sessions has not detracted from their research productivity. Although we have not done so, it would seem that practitioners could also serve as tutors with benefit to both student and physician. In our experience and in that of others who attended the Conference on Premedical Education at Buck Hill Falls in 1952, the ratio of one tutor to two students appears to be optimal. This arrangement permits greater individualization and makes for closer interpersonal relationships than would be the case with a larger number of students. Senn has described the manner in which teacher-student relationships of this type may be utilized in the teaching of the psychological aspects of medicine and the understanding of interpersonal relationships.[1] Emphasis is placed on the fact that these sessions should start promptly and that they should have precedence over any other activity except admission of new patients. The hours are arranged by appointment, and the sessions are time-limited; appointments are cancelled promptly if the student realizes that he will be unable to make the session. We believe that this emphasis on promptness and on time limitation serve to make the tutorial hour a more profitable one than would otherwise be the case.

The concept which underlies our use of the tutorial system considers the function of education and guidance to be that of helping the student to become capable of independent critical thought by making full use of his potentialities and educational opportunities. This approach, which reflects the thought behind the system of medical education at Yale, derives from a respect for the student's individuality and for his capacity to be responsible for his own growth; it attempts to stimulate his ability to learn and, thus, to teach himself. This may be accomplished through the resolution of problem situations in which the student is called upon for the application of basic information augmented by accurate and meaningful observations. When students are highly motivated and self-reliant, they may be expected to make the most of their educational opportunities; consequently, frequent examinations are not necessary. This does not deny, however, that mature students can profit from unobtrusive guidance and supervision. The tutorial program is one method of providing such guidance.

The over-all content of the tutorial sessions varies, of course, depending upon the individual tutor and students involved. Although certain topics may be discussed with each group, the sessions are generally fashioned to meet what the student's needs seem to be. These needs are usually presented in relation to patients whom the student has recently studied on the ward or whose clinical course he is currently following; they are met by helping the student develop a systematic, dynamic, and integrated approach to patient study that can be readily applied at the bedside or in the office. The emphasis in these discussions is usually on the accomplishment of early diagnosis, on the application of information from the basic sciences and medical psychology to clinical situations, on competence in history-taking and physical examination, and on a functional knowledge of growth and development—psychological as well as physical and physiological; differential diagnosis, laboratory diagnosis, and therapy may also be reviewed. The student is thus presented with diagnostic thinking that reflects scientific curiosity and interest; this is in contrast to the so-called "practical" approach that can be applied glibly and with little thought. The former approach encourages rapid growth in diagnostic acumen; the latter leads to intellectual stagnation and inferior medical care. Many of the students consider this opportunity to discuss their patients in detail with their tutor to be one of the most valuable aspects of their clerkship experience. While this is especially true for students who are just beginning their clinical years, those with previous experience in medicine and surgery gain a better understanding of the differences in approach to be used in infants and children. Another advantage of the close student-teacher relationship is the opportunity which the student has to become aware of the attitudes and feelings with which a more senior and experienced person practises pediatrics and to observe his interest in the broad aspects of child health and his relations to colleagues, children, parents, nurses, and others. These are aspects of medicine that can be transmitted only through close association, identification, and emulation.

One of the functions of the tutorial sessions is to provide students with guidance in the use of medical literature and medical libraries, to encourage them to read suitable medical journals, and to apply what they read to patients whom they are seeing; the ability to read medical literature in an efficient and critical manner will permit the student to keep informed of current advances long after the conclusion of his formal educational experiences.

For the tutorial sessions to be successful the tutor must be interested in the student and personally convinced that the sessions have real educational

value. The demands of a tutorial system require the development of new skills in teaching, since the experience of most medical instructors in this area is limited. The sessions are best if they have a personal flavor which reflects the individuality of each tutor. It is true that the tutor may overemphasize his special interests, but this is not necessarily bad. Any worthwhile teacher is enthusiastic about certain aspects of a subject, and it is this enthusiasm which may make his tutorial sessions especially valuable.

Along with the student, the tutor shares the responsibility for fostering a productive relationship. This is enhanced by an atmosphere that is permissive, friendly, and interested. As we have employed it, the success of the tutorial system depends to a great extent upon the student's initiative. For this reason, proper orientation of the student as to his rôle in the tutorial relationship is of great importance at the beginning of the clerkship. Great emphasis is placed upon the student's responsibility in this relationship. Of course, students vary tremendously in this regard. The student who is passive, disinterested, or indifferent, or who for some reason wishes to avoid this intimate relationship with a member of the faculty, gains little; on the other hand, the student who is more aggressive, interested, and able to speak freely and to say what he thinks may gain a considerable amount from this experience. Occasionally, of course, the tutor and the student who have been assigned to each other are unable to establish the desired relationship; in such cases, the value of the tutorial system becomes almost completely compromised.

Although not their chief function, a continuous process of examination unavoidably goes on throughout the tutorial periods. Actually, the tutor has a responsibility to sense the student's potentialities and to determine his current progress; otherwise, he cannot help the student to an optimal learning experience. Some tutors carry on the last session or two along the lines of an informal examination; this serves to point up the areas in which further effort is indicated.

When the tutorial system works well, it has great benefits, some of which are immediately apparent during the period of the clerkship. We have been even more impressed, however, by the fact that these relationships often persist after the student has left the clerkship. Many students continue to consult their tutors about problems which are not necessarily related to pediatrics—they may seek advice regarding interneships and residencies, about personal problems, and about patients whom they have recently seen or read about. A number of students have also elected to do their required thesis with the tutor to whom they have been assigned.

STUDENT ASSESSMENT

In addition to providing educational opportunies for the students on the clerkship, the instructional staff has an obligation to arrive at an accurate and reasonably extensive assessment of each student's performance. In addition to the use of student appraisals for record purposes and as the basis for letters of reference, we believe that the process of student assessment can promote fuller realization of a student's potentialities and contribute toward continuous improvement of clerkship teaching.

Two meetings of the attending (tutorial) and house staff are held during each clerkship period for the purpose of evaluating the performance of students; the first evaluation takes place at the end of three weeks and the second meeting at the end of six weeks. Usually, there is general agreement on the assessment of a student; occasionally, however, widely divergent opinions are expressed. If the student's performance is excellent or seems to represent his best effort, no action is taken following the first meeting; however, if the student's performance is considered to be unsatisfactory or not commensurate with his abilities, the house officers may be asked to give the student increased attention, or the instructor in charge of the clerkship may call the student in for an open discussion concerning his performance.

At the end of the clerkship each of the students is interviewed by the instructor in charge of the clerkship. Because of the number of students in each group, the interviews are, of necessity, brief and average from ten to fifteen minutes in length. These interviews serve to present to the student our assessment of his performance and permit him to verbalize any comments, criticisms, or suggestions which he may have in regard to any phase of the clerkship program. If a student has done outstanding work, he is told why we believe his performance to be superior. Although it is not as easy to communicate a less desirable assessment, this can usually be accomplished with the student's acceptance and concurrence. Students appreciate that a conscientious effort has been made to arrive at a fair appraisal; they recognize that the policy of calling attention to their deficiencies reflects a real interest in their personal welfare. On rare occasions a student disagrees with our assessment; in this event, the appraisal is reviewed with each member of the staff. In those instances in which our appraisal of a student is uncertain, a definitive opinion is not given until conclusion of the senior clerkship. The response of the students to this utilization of their assessment has been very favorable; that they are appreciative of hearing what others think about them is reflected by both their verbal and nonverbal responses during the interview.

In addition to providing the student with an evaluation of his performance, the interview permits the instructional staff to obtain a frank appraisal of the value of the clerkship from the student's viewpoint. Comments and suggestions which the students offer are used to modify and improve current teaching practices.

Medical students, like other persons, occasionally have psychological symptoms (anxiety, depression, learning difficulties, inability to study, etc.) which severely affect their happiness and handicap their effectiveness. Often these reflect transient and situational problems; occasionally, however, they are manifestations of severe and long-standing problems. It would seem to be a function of the instructional staff to recognize the presence of these problems and to assist the student in obtaining professional help. We believe this to be an important aspect of the assessment program.

SUMMARY

Superior and inspired teaching derives best from a close relationship based on mutual respect between student and teacher. Frequently, the student feels that no one on the faculty really knows him or is interested in his individual welfare. This state of affairs would seem to indicate the need for a greater opportunity for students and instructors to get to know each other better. This paper describes an attempt to meet this need by the use of a tutorial system and personal interviews.

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EPIDEMIOLOGY OF INFECTIOUS HEPATITIS IN AN URBAN POPULATION GROUP

Report on an Epidemic in a Medium-Rental Housing Project in New Haven, Connecticut in 1953**

Until the past ten years, the spread of infectious hepatitis in civilian populations has not been so extensively analysed as in military groups.[6, 7, 13] The reports on this disease among civilians have mainly been concerned with outbreaks either in rural districts,[4, 9, 15] or in institutions.[1, 2, 8] However, an epidemiological review of hepatitis with comparisons of its rural and urban situations in close-by towns and villages in East Anglia for the period 1943-47 has been included among its other aspects in a Special Report of the Medical Research Council of Great Britain.[12] The observations of Capps, Stokes, and their associates,[1, 2, 8] who have described outbreaks in institutions, indicated the definitive rôle of children and infants as carriers and the control of its spread to nurses through simple improvement of the nursing techniques, which also further confirmed the importance of the transmission by fecal-oral route.[13] Recently, an outbreak in a low-rental housing project in Baltimore, Maryland, was described.[11] Actually, however, relatively little data have been collected in the United States in regard to the epidemiological and endemiological aspects of hepatitis in urban populations.

INFECTIOUS HEPATITIS IN NEW HAVEN, CONNECTICUT

This disease became reportable in the city of New Haven, Connecticut, in 1948. The annual reported incidences since then are listed in Table 1. During the first three months of 1953, over 50 cases were reported in this city. During that period, there seemed to be a concentration of cases mainly

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in two municipal housing projects, viz., 9 cases in a medium-rental and 7 in a low-rental housing project. A family-to-family survey at the former housing project was conducted by the authors during the week of March 30 through April 4, 1953. This was supplemented by questionnaires and visits in May 1953.

The purpose of this study has been to review the epidemic situation in this medium-rental housing project* with a view to finding possible causes leading to the local concentration of cases there.

TABLE 1. ANNUAL REPORTED INCIDENCES OF INFECTIOUS HEPATITIS IN THE CITY OF NEW HAVEN, CONNECTICUT

Year	*Number*
1948	0
1949	1
1950	0
1951	3
1952	5
1953 (Jan. through May)	109

TOPOGRAPHY AND GENERAL SANITATION OF THE SURVEYED HOUSING PROJECT

The housing project was located on the outskirts of the City of New Haven (in Census Tract 13, see Fig. 1). There were only a few inhabited dwellings in the immediate vicinity. A fair number of other houses were about one-half to one mile away, but with no direct connecting roads. Thus, the housing project was a more or less isolated community at that time.

There was a swamp on the eastern border of the premises with a pond at its south end (Fig. 2). A small creek ran from north to south on the western border of the grounds. On the other side of the creek were the remains of abandoned pig pens. A small group of trees served as a fence on the north. The only entrance to the grounds was at the south end.

The water supply was from a common main of the New Haven Water Company. The sewerage system was provided by the city. Garbage was disposed of by an electric disposer in the kitchen of each apartment. Other refuse was collected in covered bins in the back of the building. The rental was considered of medium class. The general sanitation and personal hygiene of the families was good.

* This will hereafter be referred to merely as "the housing project."

CHARACTERISTICS OF THE POPULATION SURVEYED

Two hundred and fifty-four families, the heads of which were mainly skilled artisans who worked in various parts of the city, lived in apartments within the housing project composed of 33 brick buildings (Fig. 2). All the

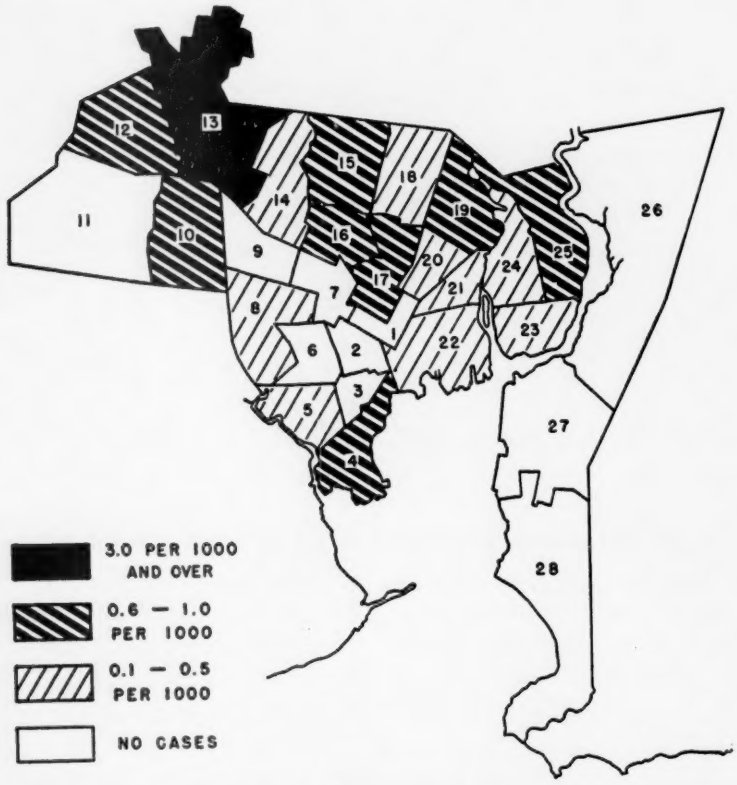

FIG. 1. Notification rates of infectious hepatitis in the city of New Haven, Connecticut, January through May 1953, according to census tracts.

buildings were new and most of them had been inhabited for less than 20 months.

Visits to 252 of these 254 families were made by the authors during the week from March 30 through April 4, 1953. Two families were not available at the time.*

* According to the information obtained from the Manager's Office of the housing project, there were four adults and three children in these two families. No hepatitis case occurred among them so far as we know. Their exclusion did not materially affect the statistics.

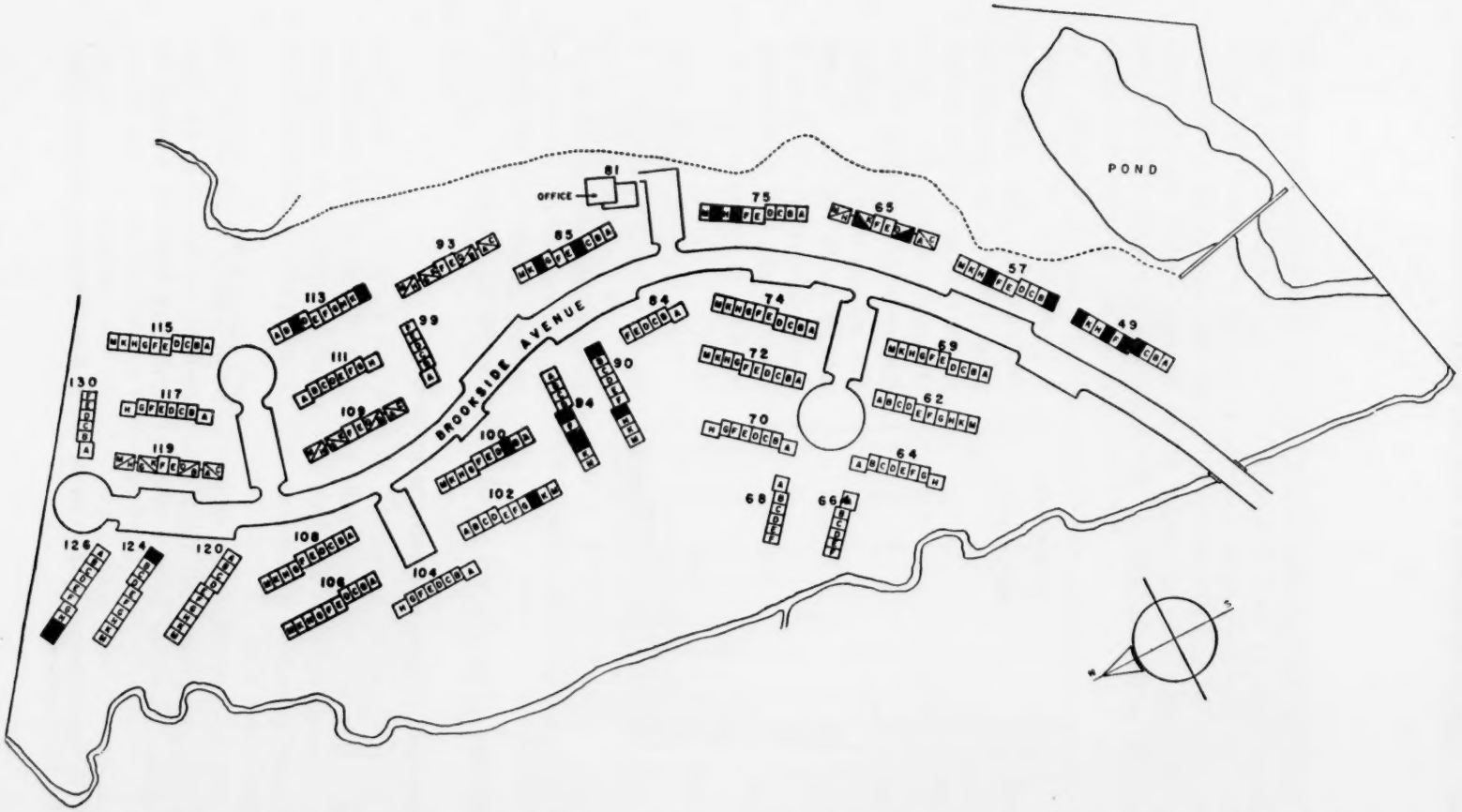

FIG. 2. Topography of the housing project. Black areas indicate the families with patients. The numbers correspond to the buildings. The letters correspond to the apartment spaces.

These 252 families consisted of 1,160 residents, 556 being adults and 604 children (aged 14 years or younger). The ratio of children to adults was 1.1 : 1 (or four times higher than that for the city as a whole*). This high proportion of children was due to the fact that one of the prerequisites for the rental of an apartment in the housing project was that there should be at least one child in the family. The age distribution is shown in Figure 3, with two major groups, one at the ages 0 to 9 years (45% of the population) and the other at 20 to 39 years (36% of the population). All the families had lived in New Haven and nearby towns before they moved to the housing project. The average number of individuals per family was 4.6. The frequency distribution was shown in Figure 4, with 80% of them having three to five individuals in a family.

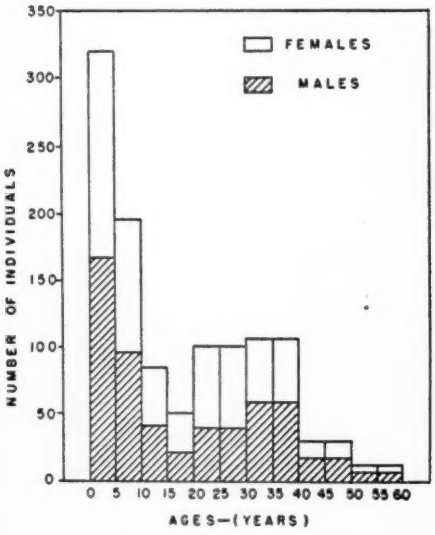

FIG. 3. Age distribution of the surveyed population.

There were no schools nearby. The children were taken to and from the schools by school buses, which were usually crowded. Communication between the housing project and the downtown area of the city was provided by a special bus route.

DIAGNOSTIC CRITERIA OF INFECTIOUS HEPATITIS

When the survey started, the epidemic was approaching its end. Only three acute patients were actually seen by us. Therefore, the diagnosis of all the remaining cases was done in retrospect by carefully questioning the convalescent patient, if he was an adult, or the parents, if the convalescent patient was a child.

Only those patients with a definite history of jaundice in addition to other pertinent symptoms and signs were considered to have had infectious hepatitis. Inasmuch as the colors of urine and stools as assessed by

* The ratio of children to adults for the City of New Haven as a whole was 0.27 : 1 in the 1950 census.

the parents were hard to depend on, those non-icteric patients with "dark urine and light-colored stools" were not included in our study unless there was at least one icteric patient in the same family or among the close contacts. We were compelled to do so because the prevalence of "flu" and minor illnesses during the same period among the inhabitants made our appraisal of their statements rather difficult. Therefore, most of the non-icteric cases were excluded by such a selection.

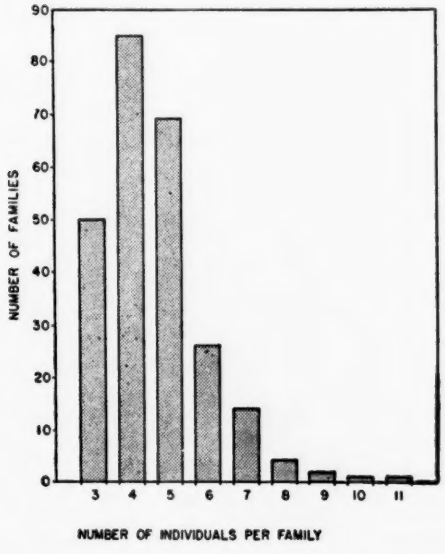

FIG. 4. Frequency distribution of families according to number of individuals per family.

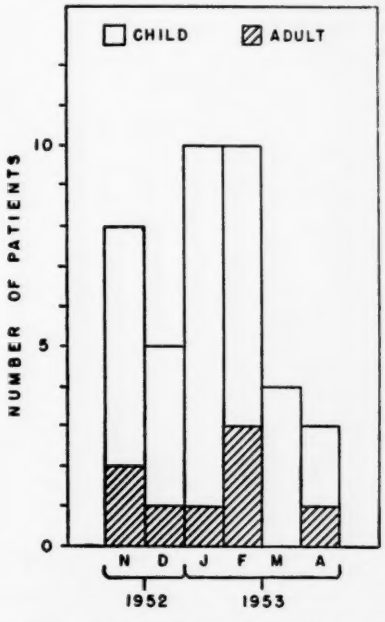

FIG. 5. Monthly distribution of patients.

From our survey, there were possibly 10 to 15 suspected (non-icteric) patients in addition to the icteric, giving a ratio of non-icteric and icteric as about 1 : 3, similar to that calculated from the reports by Davis and Hanlon,[4] and by Ipsen *et al.*[9]

THE EPIDEMIC IN "THE HOUSING PROJECT"

The first two cases occurred more or less coincidentally in the early part of November 1952 in buildings No. 124 and 94 (see map in Fig. 2). By the middle of that month, four more cases appeared in buildings No. 90 (2 cases), No. 65 (1 case), and No. 57 (1 case). From then onwards, more cases cropped up with a total of 40 before the end of April 1953. The

majority of cases were mild. None of them had a relapse. There were no fatalities. Not all the cases were seen by physicians. Only one was hospitalized.

1. *Monthly incidence* (according to the date of onset). As shown in Figure 5, the epidemic seemed to start in November, with eight patients in that month, although from the shape of the graph one might suspect that undetected cases had occurred prior to November 1, 1952. There was a

TABLE 2. AGE AND SEX DISTRIBUTION OF PATIENTS

	Male			*Female*			*Total*		
Ages (Yrs.)	*No. of individuals*	*No. of patients*	*Rates per 1,000*	*No. of individuals*	*No. of patients*	*Rates per 1,000*	*No. of individuals*	*No. of patients*	*Rates per 1,000*
Under 1	24	0	0	26	0	0	50	0	0
1-4	145	7	48	128	3	23	273	10	37
5-9	96	8	83	99	7	71	195	15	77
10-14	42	3	71	44	4	91	86	7	81
15-19	22	1	45	29	1	35	51	2	39
20-29	81	0	0	121	4	33	202	4	20
30-39	121	1	8	93	1	11	214	2	9
40-49	36	0	0	25	0	0	61	0	0
50-60	17	0	0	11	0	0	28	0	0
Total	584	20	34	576	20	35	1,160	40	34
0-14	307	18	59	297	14	47	604	32	53
15+	277	2	7	279	6	21	556	8	14

peak in January and February 1953. By March 1953 the incidence was declining, with no cases in May 1953. Because the survey was done in retrospect, most of the patients could not recall the exact date of the onset of their illness. A charting of the incidence by week was not possible.

2. *Morbidity rate and age distribution.* There were 40 cases among 1,160 inhabitants, with a crude morbidity rate of 34 per 1,000 (Table 2).

The youngest patient was 18 months old. The age specific morbidity rate reached its peak at the age of 5 to 14 years (Table 2 and Fig. 6). Thereafter, the rate rapidly declined. The oldest patient was aged 35 years. Judging from the age specific morbidity rates, the most vulnerable age seemed to be between 5 and 15 years.

Among the *children* (aged 0 to 14 years), 32 out of 604 (or 53 per 1,000) had the disease, in contrast to the adult rate in which only 8 out of

556 *adults* (or 14 per 1,000) were affected. The difference in the morbidity rates between children and their parents was statistically significant ($\chi^2 = 12.95$, p = 1 : 6,000 approximately) (Table 2).

It should be noted that the lack of patients under the age of one year was very likely attributable to the presence of *only* one infant (7 weeks old) among the 24 affected families from which these 40 cases came.

3. *Sex incidence.* Incidence among the boys and girls was not statistically different (viz., 59 per 1,000 and 47 per 1,000, respectively). Of the 8 sick

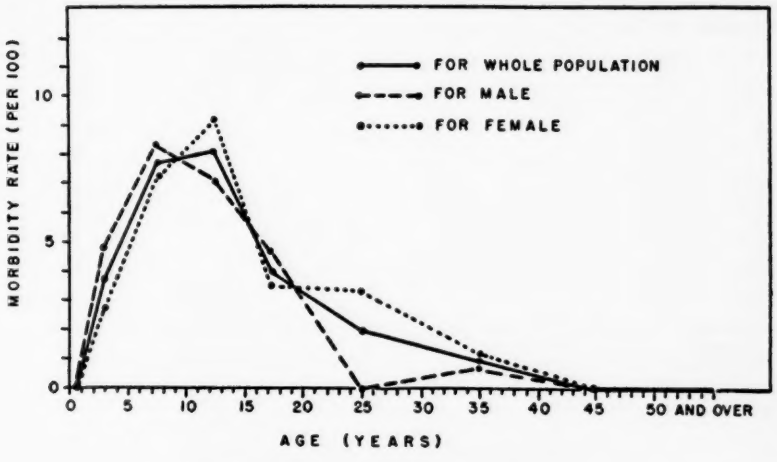

FIG. 6. Age and sex specific morbidity rate.

adults, there were 6 females (including 5 mothers) and 2 males (1 father) (Table 2). Statistically, this difference could have also been due to chance. Nevertheless, the apparent difference* between the number of sick mothers and sick fathers (viz., 5 and 1, respectively) led one to wonder whether it was because of closer contacts between the children and mothers than the fathers, who were away most of the day. That is to say, this disease could possibly be considered an "occupational hazard" to the mothers. This apparently higher incidence among mothers as opposed to fathers was comparable to that noted in the family studies on respiratory diseases by Dingle with two major groups, one at the ages 0 to 9 years (45% of the population) and the other at 20 to 39 years (36% of the population). All the

* For the occurrence of 5 sick mothers and 1 sick father among 252 pairs of parents, $\chi^2 = 2.7$ and p = 1 : 10. The difference was not, actually, statistically significant.

families had lived in New Haven and nearby towns before they moved to the housing project. The average number of individuals per family was 4.6. The frequency distribution was shown in Figure 4, with 80% of them having three to five individuals in a family.

There were no schools near-by. The children were taken to and from the schools by school buses, which were usually crowded. Communication and his associates[5] where, as in hepatitis, by far the largest rates also occurred in the children.

TABLE 3. DISTRIBUTION OF PATIENTS IN VARIOUS BUILDINGS ACCORDING TO MONTHS

Bldg. no.	*Nov.*	*Dec.*	*Jan.*	*Feb.*	*Mar.*	*Apr.*	*Total*
49			2	4	3	3	12
57	1		1				2
65	1	3					4
75	1			3			4
85		1	1				2
90	2			1			3
94	1		2	1			4
100			1				1
102		1					1
113				1	1		2
124	1						1
126	1		3				4
Total	8	5	10	10	4	3	40

4. *Topographical distribution.* The patients were distributed in 12 buildings, which could be grouped as follows (Fig. (2):

(a) Buildings Nos. 49, 57, 65, 75 : 22 patients
(b) " " 90, 94, 100, 102 : 9 "
(c) " " 124, 126 : 5 "
(d) " " 85 : 2 "
(e) " " 113 : 2 "

The first two cases at the beginning of the epidemic were located in Buildings Nos. 94 and 124. The next six cases in November were in Buildings Nos. 57, 65, 75, 90, and 126 (Table 3). From these foci, the infection spread out in the next five months. The building with the most of the cases was No. 49, where 12 cases occurred in four families. The cases

were tabulated in Table 3, according to the date of onset and the location of the buildings.

It should be noted that in three instances, cases occurred in adjacent families in the same buildings (viz., 49 E and D, 94 G and H, and 126 K and M; see map in Fig. 2). In another three instances, cases appeared in adjacent apartments in two neighboring buildings (viz., 49 M and 57 A, 90 G and 94 G, and 100 C and 102 H; see map in Fig. 2).

5. *Multiple cases in patients' families.* Of the 252 families visited, 50 had three members (i.e., two parents and *only one* child). None of these 50 (single-child) families had anyone sick with this disease during that period.

TABLE 4. FREQUENCY DISTRIBUTION OF FAMILIES WITH PATIENTS ACCORDING TO THE TOTAL NUMBER OF INDIVIDUALS IN FAMILY

Total no. of individuals in family	*No. of families visited*	*Families with patients*	
		No.	*%*
3	50	0	0
4	85	6	7
5	69	11	16
6	26	4	15
7	14	2	14
8-11	8	1	12
Total	252	24	9

From 7% to 16% of the remaining families with four or more individuals (i.e., parents and *two or more* children) had one or more cases (Table 4*).

The 40 cases at the housing project came from 24 families (or 9.5% of the 252 families visited). Of these 24 families, 16 had only one case in their families and eight had more than one case (Table 5*). There was an apparent tendency for more cases to occur in larger families (Table 5).

Sometimes, the multiple cases in the same family had their onset on the same date. There were four such families. In most of the families, the exact date of onset could not be recalled. Therefore, a calculation of the secondary attack rate was not feasible.

* In Tables 4 and 5, the heading of "total number of individuals in family" was used instead of "number of chidren," because in several big households there were, in addition to those of 14 years or younger, older children who were classified as adults in this study.

Of the 40 cases here, 12 could give the definite date of onset and a clear description of their disease. These patients were arranged as follows in chronological order according to the date of onset of their disease:

Name	*Age (years)*	*Date of onset*
Wayne F.	4	Jan. 20
David S.	10	" 30
Richard S.	6	Feb. 1
Mrs. F.	27	" 15
Paul F.	10	" 15
Barry F.	1½	" 15
Carol C.	12	Mar. 24
Barbara S.	7	" 27
Bob Q.	2	" 30
Sharon A.	5	Apr. 3
Mrs. Q.	26	" 13
Howard C.	10	" 14

TABLE 5. FREQUENCY DISTRIBUTION OF FAMILIES WITH PATIENTS ACCORDING TO THE TOTAL NUMBER OF INDIVIDUALS IN FAMILY AND THE NUMBER OF PATIENTS IN FAMILY

Total no. of individuals in family	*No. of patients in family*					*Total*
	1	*2*	*3*	*4*	*5*	
4	4	2				6
5	8	1	2			11
6	3			1		4
7	1		1			2
8–11					1	1
Total no. of affected families	16	3	3	1	1	24
Total no. of patients	16	6	9	4	5	40

The F., S., Q., and A. families lived in the same building and were close friends. The two children of the C. family were half-siblings of the S. children. Though they did not live regularly on the premises, they stayed with the S. family very frequently and often for long periods of time.

It seemed possible that the first three children (Wayne, David, and Richard) became infected at about the same time. However, they could not recall that any of their playmates, schoolmates, or other contacts had been sick with jaundice before they developed the disease. From Wayne F. the

disease was transmitted to his mother and two of his siblings with a lapse of 25 days. The secondary case in the S-C household did not appear until 52 days after David and Richard, or 37 days after Mrs. F. and her two children. Bob Q., Sharon A., Mrs. Q. and Howard C. might be considered tertiary and tetrary cases.

THE EPIDEMIC SITUATION IN THE CITY

As shown in Table 1, the reported incidence of infectious hepatitis had been low in the preceding years. The five cases reported in 1952 occurred one each in January, March, June, October, and November, scattered in various parts of the town. The case in November 1952 was a resident of the housing project and included in our analyses.

When the hepatitis cases appeared in the housing project, there were also cases reported in other parts of the city. During the period covered by our survey (i.e., November 1952 through May 1953), a total of 109 cases were reported in the city of New Haven.* Of these 109 patients, 10 were reported by practising physicians as being residents at the housing project and 30 more were found there by us. The discrepancy between the number of reported cases and that of the existing cases in the housing project was probably due to the fact that all the patients were not seen by physicians. If this ratio of 1 : 4 of reporting would hold true for the city as a whole, there should have been about 300 cases** in the city during that period.

An analysis of the distribution of the 109 cases in the city is shown in Figure 1. The notification rate of infectious hepatitis for the period from November 1952 through May 1953 for the city as a whole was approximately 0.6 per 1,000 population. The city was divided into 28 census tracts. Of these, 9 tracts had notification rates above 0.5 per 1,000, 9 had rates between 0.1 and 0.5 per 1,000, and 10 had no cases during that period (Fig. 1). Actually, the rate for the census tract (No. 13) where the housing project was located was much higher than elsewhere in the city; viz., 4.0 per 1,000 if the 30 extra cases which were found by our survey were not included, or 10.3 per 1,000 if they were included (Fig. 1).

DISCUSSION

Our data on this epidemic should be examined in the light of the following facts:

---

* From June through November 1953, two more cases were reported at the housing project and another eleven elsewhere in the city.

** The estimate for the city was based on the assumption: $4 \times (109 - 30) = 316$.

(a) the local population had a preponderance of youngsters below the age of 15 years;

(b) the population was distinctly urban. Contacts between neighboring families, and their children in particular, were close;

(c) only jaundiced patients were counted during the family-to-family canvassing;

The aggregation of children (45% of the residents) in the housing project meant enhancement of the mass susceptibility of the population to infectious hepatitis. This not only exaggerated the morbidity rate but perpetuated the epidemic there. The mild nature of the disease often seen among the children further favored its spread because such cases are commonly missed—those old enough to get about are ambulatory and thus serve as carriers of the infection.

The first cases in the housing project occurred more or less simultaneously. These patients claimed that they had not been in contact with any jaundiced patients in the previous two months before they became sick and that they had no close association among one another though there was little doubt that cases could have existed in October 1952 or earlier.* Therefore, the origin of their infections was indeterminate. Among subsequent cases, six might possibly have been the sources of infection to 18 others. The rest either could not give a definite history of contacts or claimed no such similar disease among their friends. However, it was our impression after talking with the people there that the contacts among children were far more frequent and closer than we had anticipated. In view of the probable frequency of unrecognized subclinical cases, particularly in children, efforts to trace case-to-case spread of this disease would not be rewarding.

Contaminated water supply was considered as a possible means of spread but the evidence was not in favor of such a hypothesis. Firstly, the topographical distribution of cases did not quite correspond to the water system. Secondly, the epidemic was not as "explosive" as the usual water-borne type. Thirdly, the plumbing was new and a cross connection between the sewer and the water pipes was considered unlikely by the sanitary authorities.

All the families used pasteurized milk from three different suppliers. The patients were more or less evenly distributed among the three suppliers.

Though there were only 49 dogs and 14 cats registered, there were a fair number of stray dogs on the grounds. That "canine viral hepatitis" could

* The epidemic season for infectious hepatitis in New Haven has not been determined, but on the basis of local experience in 1944-45, cases began to increase in October and decline in January and February.[14]

have been the source of the infection was a remote possibility. Nevertheless, sera were obtained in May 1953 from 46 residents at the housing project: 13 from individuals who had the disease during the epidemic, 13 from family contacts, and 20 from non-contacts. Complement fixation test[10] was performed, using the liver from a dog suffering from canine hepatitis as antigen.* None of the human sera gave a positive test.

SUMMARY

In the winter and early spring of 1952-53, there was an epidemic of infectious hepatitis in New Haven, Connecticut. There was a concentration of cases in several census tracts. One housing project (medium-rental) in the census tract with the highest incidence was surveyed.

Of the 254 families on the premises of this housing project, 252 were visited. The total population was 1,160 with 556 adults and 604 children (i.e., a ratio of 1 : 1.1). There were 40 patients (32 children and 8 adults) with unmistakable symptoms and signs of infectious hepatitis from November 1952 through April 1953. The disease was significantly more prevalent among the children than among the adults. The most vulnerable age (with highest age-specific morbidity rates) seemed to be between 5 and 15 years. There was no predilection to either of the sexes. It seemed that the disease was spread through close contacts, particularly of children, and that the high local incidence was influenced by the large susceptible population of children which was a special characteristic of the housing project studied.

Sera were obtained from patients, contacts, and non-contacts. None gave a positive complement fixation test with a sample of canine hepatitis CF antigen.

ACKNOWLEDGMENT

Gratitude is particularly due to Professor John R. Paul for his guidance and counsel in this study, and to Mr. N. M. Lavin who generously gave us the canine hepatitis complement fixing antigen and antiserum.

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THE PERCENTAGE OF TAPERED FIBRIL ENDS IN SKIN COLLAGEN FROM CASES WITH AND WITHOUT "COLLAGEN DISEASE"

Many methods have been used in an effort to elucidate any change that may occur in collagen plus ground substance in the group designated "collagen disease." The electron microscope furnishes fine morphological detail, and it was hoped that a correlation could be established between the configuration of the collagen fibril ends and the type of disease, which would shed light on the processes involved. Banfield[1] has already shown that the number of fibrils with tapered ends, in contradistinction to those with square or broken ends, is increased where collagen is apparently being actively formed (embryonic human tissue and mesenchymal neoplasms). The possibility of similar collagen activity in diseases of the connective tissue prompted the present study.

The abdominal skin collagen from 104 individuals (20 from biopsies and the remainder from autopsies) with and without collagen disorders were examined under the electron microscope, and the percentage of tapered to total fibril ends determined.

MATERIALS AND METHODS

Abdominal skin from the left upper quadrant obtained from biopsy or autopsy was stored unfixed in the frozen state. After thawing, 90-120 mg. (wet weight) of dermis was minutely dissected from epidermis and subcutaneous fat and homogenized in 150 ml. demineralized water in a Waring blender in the cold room for 15 minutes. The final temperature of the solution was 30° C. or less in each case. The cup was washed out with an additional 20 ml. of demineralized water and drops taken from the resulting suspension with standard Kimble glass melting point capillary tubes, a fresh tube being used for each specimen. The drops were placed on collodion-covered 200 mesh Cu-Ni electron microscope grids, allowed to dry, washed for 15 minutes in demineralized water, shadowed with palladium, and examined in the RCA Model EMU 2A electron microscope. Duplicate grids were scanned and the number of tapered and square or torn fibril ends were counted and recorded on a differential blood count adding machine. Fibril narrowings and tactoids (short lengths of collagen fibrils tapered at both ends) were also recorded, the latter being counted as two tapered ends in the calculation. Consecutive fields were carefully examined and 200 or more non-tapered ends were counted per grid, making a total of 400-450 fibril ends per sample of skin.

* Arthritis and Rheumatism Foundation Research Fellow.
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In a few cases the collagen was scanty, so that only 100 or more ends could be counted per grid. Specimens obtained from 104 different individuals were examined.

Owing to insufficient skin, the dermis of a few specimens weighed only 50-90 mg. However, preliminary investigations on different weights of the same autopsy dermis showed no significant alteration in the counts obtained from a fairly wide variation in

TABLE 1. AVERAGE % TAPERED TO TOTAL COLLAGEN FIBER ENDS (SEE TEXT). THIRTY-TWO CASES OF "COLLAGEN DISEASE" AND 72 CASES DYING FROM OTHER CAUSES

Disease causing death	*No. of cases*	*Average reading*	*Extreme readings*
	"Collagen diseases"		
Dermatomyositis	1	2.9	.....
Scleroderma	1	6.8	.....
Spondylitis	1	4.8	.....
? Polyarteritis nodosa	1	5.4	.....
Diss. lupus erythematosus	7	3.1	1.0–7.1
Rheumatic fever	1	4.2	.....
Rheumatic heart disease	8	3.2	1.0–5.0
Rheumatoid arthritis	12	3.6	1.0–5.2
	"Non-collagen diseases"		
Prematurity and stillbirth	6	4.9	1.5–11.2
Congenital defects	12	3.8	1.4–7.8
Leukaemia and lymphoma	7	3.8	1.5–8.2
Cardiovascular disease	14	4.0	1.5–8.0
C. N. S. disease	2	4.7	4.1–5.3
Neoplastic disease	6	3.6	2.8–4.0
Icteric cases*	8	4.7	1.5–6.6
Infections	2	4.2	1.9–6.6
Miscellaneous	7	3.6	1.2–5.7
Accident	8	3.8	1.9–5.6

* The icteric cases included carcinomata of the pancreas, gall bladder, and ampulla of Vater, impacted gallstone, hepatic cirrhosis, and a lymphoma involving the pancreas with jaundice.

wet weight (60-120 mg.) and blending time (5, 15, and 30 minutes). By using a measured volume of water and standard-width capillary tubing throughout, variations in suspension concentration and deposition of fibers on the capillary walls were kept to a minimum.

RESULTS

1. *Quantitative.* As shown in Tables 1 and 2, there was *no* significant difference in the per cent of tapered to total (i.e., tapered and square or

torn) fibril ends regardless of age, type of disease, disease activity or therapy. All readings fell between 1 and 8% except for one premature still birth which was 11.2%. The samples from three other premature infants contained 1.5, 3.7, and 4.9% tapered ends respectively. All cases except one over the age of 50 years gave readings above 3%. Examples of "collagen disease" gave counts similar to those from individuals dying from a variety of causes. Samples from cases with active rheumatoid arthritis with acutely inflamed joints and fulminating rheumatic heart disease did not differ significantly from others with subacute or inactive rheumatoid arthritis or

TABLE 2. AVERAGE READINGS BY DECADES (SEE TEXT). THIRTY-TWO CASES OF "COLLAGEN DISEASE" AND 72 CASES DYING FROM OTHER CAUSES

	Age in years									
	under 1	*1–10*	*11–20*	*21–30*	*31–40*	*41–50*	*51–60*	*61–70*	*71–80*	*81–90*
					"Collagen diseases"					
No. of cases	..	1	2	7	6	6	8	1	1	..
Average Reading	..	2.9	3.0	2.8	3.6	4.3	3.6	5.4	5.0	..
					"Non-collagen diseases"					
No. of cases	15	10	3	8	4	7	7	8	7	3
Average Reading	4.7	3.5	3.6	2.6	3.5	3.1	4.4	4.4	5.0	5.1

long-standing rheumatic cardiac decompensation, in respect to the relative number of tapered ends. Concurrent treatment with cortisone, ACTH, phenylbutazone, or aspirin did not produce any significant alteration in the result.

One case of acute pancreatitis and three cases of diabetes mellitus of 11-20 years' duration were included in the "miscellaneous" group in Table 1, and a carcinoma of the pancreas without jaundice was put in the "neoplastic" group. The icteric cases included carcinomata of the pancreas, gall bladder, and ampulla of Vater, impacted gallstone, hepatic cirrhosis with and without pancreatitis, and a lymphoma involving the pancreas and producing jaundice. However, all of these pancreatic conditions (inflammation, new growth, atrophy, or fibrosis) gave similar quantitative results.

A few specimens, examined shortly after removal, had been kept at 4° C. only. These gave identical morphology and similar counts to the majority of specimens which had been stored frozen for 1-12 months. One man aged 22 years died with acute rheumatic heart disease. Comparison of counts

made from biopsy skin within a week of death and autopsy skin from the same region, showed no significant difference (2.5 and 1.4% respectively: a total of 620 square ends recorded). One nine-year-old boy dying of dermatomyositis gave a count of 2.9% in macroscopically normal abdominal skin and 4.2% in rash-bearing skin from the upper outer chest (a total of 705 square ends recorded).

2. *Qualitative.* Although the various pancreatic conditions listed above gave similar quantitative results, nearly all cases exhibiting jaundice, from whatever cause, showed a qualitative change in the type of tapered end. These ends were long and filamentous (Fig. 1) and occurred in seven out of the eight icteric individuals. Similar filamentous fibril ends were also seen in seven non-icteric cases of predominantly connective tissue disease: one adult with scleroderma, one with disseminated lupus erythematosus, one with multiple sclerosis, and one with chronic rheumatic heart disease; an eighteen-year-old boy with acute rheumatoid arthritis, a seven-year-old child with acute leukaemia, and a month-old baby dying of congenital heart disease.

Table 3 analyses the cases showing fibril narrowings and tactoids (short lengths of collagen fibrils tapered at both ends) noted during the examination. Samples from 28 individuals of all ages and suffering from a variety of conditions exhibited these changes which are identical with those found following the action of collagenase and trypsin on collagen *in vitro*[4] (Fig. 1). It so happened that seven of these 28 cases also had filamentous fibril ends, being part of the total recorded above that showed this qualitative change. Two of the individuals with the greatest number of fibril narrowings (one with carcinoma of the pancreas and one with carcinoma of the gall bladder) were both icteric.

3. *Epidermis.* In two cases (aged 62 and 75 years) the epidermis remaining after dissection of the dermis (including the attached papillary layer of the dermis) was treated in the same way and duplicate grids were counted. The percentage of tapered ends was seven and four, respectively, and did not differ significantly from the results found in the true dermis from the same samples of skin. Four fibril narrowings seen in the first and one in the second case were identical to those listed in Table 3.

DISCUSSION

Banfield[1] showed that the number of collagen fibrils with tapered ends was greater in sites where collagen was apparently being actively formed (embryonic human tissue and mesenchymal neoplasms) than in adult

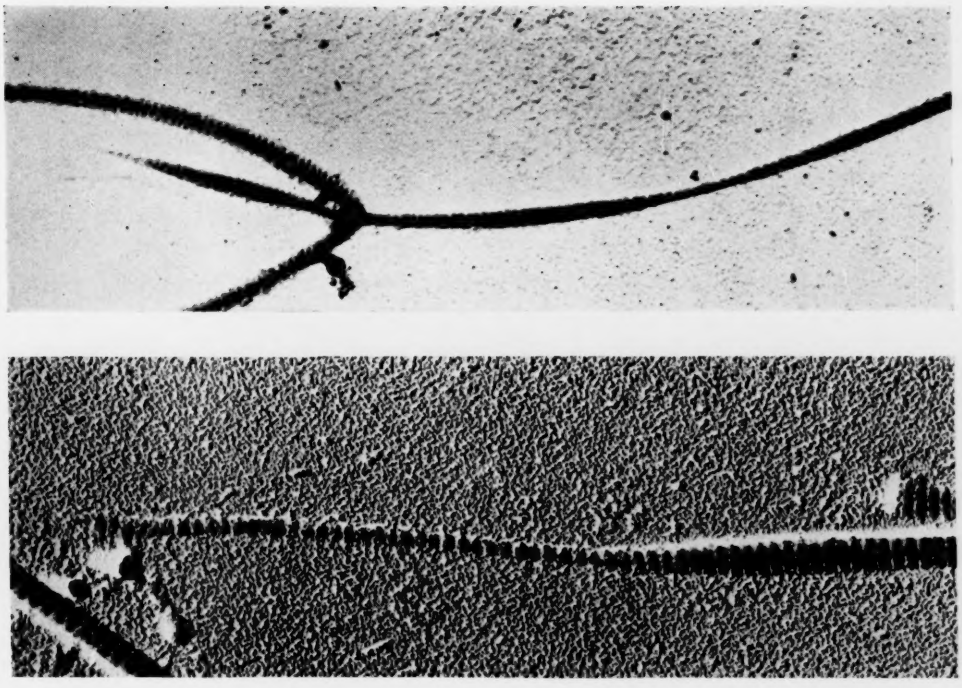

FIG. 1. *Top:* Collagen fibril showing fibril narrowings and a tapered end from the abdominal skin of an icteric 74-year-old man dying of carcinoma of the gall bladder. Palladium shadowed. x 18,500

Bottom: A filamentous fibril end from a 49-year-old man dying of chronic rheumatic heart disease. Palladium shadowed. x 28,000

"resting" connective tissue. Recent quantitative studies[2] have shown a very high percentage of tapered ends in the fibrils of skin of young human embryos. This decreases markedly by the time of birth. In the present study (Table 2) the average reading for 15 babies under the age of one year was 4.7%. Recent calibration studies of fibrils from embryonic skin[2] and Achilles tendon[5] show that the fibrils are narrower in embryos and babies than in adults. This was confirmed by direct observation. Although the grids to be examined had been labelled anonymously by another individual, collagen from infants and young children could be easily recognized by the higher magnification required for fibril counting as well as by the greater quantity of amorphous material present. This increase in amorphous material (ground substance) in preparations of infant as opposed to adult skin has been noted by Gross[3] and Pahlke.[5] In the present investigation it appeared to cause clumping and looping of the collagen fibrils so that the fiber ends were scanty in the presence of plentiful collagen. This fact might possibly produce a falsely low count in children under five, although it is difficult to prove that tapered ends are more liable to be obscured than cut or torn ends.

Collagen is the principal extra-cellular fibrous protein occurring in the connective tissue of higher animals, forming 20-30% of the total body protein. In spite of bearing the brunt of the attack in the varied disorders termed "collagen disease," demonstration of altered collagen fibrils from affected areas by electron microscopy has proved difficult. However, changes in senile elastosis and some rare skin conditions were reported by Tunbridge,[6] and Rich[6] recently described markedly abnormal collagen fibrils in rabbit skin at the site of experimentally produced local anaphylactic lesions of the Arthus type. Stoughton and Lorincz[7] produced evidence that normal serum albumin has a definite anti-collagenase action as measured histochemically in acetone-fixed sections of human skin, using filtrates of *Cl. welchii* Type A. This anti-collagenase activity was confined to the albumin fraction, was destroyed by heat, and was decreased in one case of dermatomyositis and three patients with subacute lupus erythematodes as compared with sera of five normal adults. It is suggested that this decrease might be quantitative rather than qualitative, due to the well-known lowered serum albumin in these diseases. However, the authors, in attempting to explain the significance of this anti-enzyme action, postulate that in disease there is an imbalance of a normal collagenase- anti-collagenase equilibrium. As yet, no human or animal source of collagenase has been demonstrated, but previous work[4] suggested the presence of an enzyme

"contaminant" associated with highly purified trypsin* obtained from bovine pancreas. This "contaminant" acted on human skin collagen giving the same electron microscope picture as collagenase. Further evidence that people of all ages possess some substance in the dermis capable of altering

TABLE 3. ANALYSIS OF FIBRIL NARROWINGS AND TACTOIDS SEEN IN 28 OF THE 104 CASES EXAMINED (SEE TEXT)

P.M. No.	*Age*	*Diagnosis*	*FN*	*T*	*E*
10,035	NB	Prematurity	1	..	306
9700	NB	Stillbirth	1	..	432
10,019	1 D	Multiple congenital deformities	1	..	313
10,070	2 D	Congenital heart	2	..	416
St.F. 53-118	13 M	Acute leukaemia	1	..	214
B. 53-113	7	Acute leukaemia	1	..	404*
H.H. 8231	11	Infectious neuronitis	1	..	404
D.C. (B)	18	Acute rheumatoid arthritis	1	..	416*
H.H. 8190	22	Accident	1	..	408
N.J. (B)	25	Rheumatic heart disease	..	1	418
H.H. 8196	27	Brain tumor	1	..	412
A.G. (B)	41	Subacute rheumatoid arthritis	1	1	408
M. 83-53	41	Idiopathic myocarditis	2	..	400
10,026	49	Rheumatic heart disease	1	..	414*
10,031	49	Lymphocytic lymphoma	1	1	433
G.S. (B)	49	Scleroderma	1	..	420*
10,098	51	Ruptured aortic aneurysm: hypertension	2	1	406
L.G. (B)	51	Subacute rheumatoid arthritis	..	1	422
9775	54	Cirrhosis and jaundice	1	..	310
M.H. (B)	57	Acute rheumatoid arthritis	1	..	435
10,029	58	Multiple sclerosis	..	1	440*
10,179	67	Coronary thrombosis	1	..	452
10,082	69	Coronary thrombosis	1	1	411
9905	69	Ca. of pancreas without jaundice	4	..	405
9771	70	Diabetes mellitus for 11 years	1	..	301
10,105	72	Hypertension	2	1	412
10,050	74	Ca. of gall bladder with jaundice	6	2	401*
9910	86	Ca. of pancreas with jaundice	3	..	408*

FN—fibril narrowings T—tactoids E—number of square ends counted
NB—newborn D—days M—months (B)—biopsy *—filamentous fibril ends

*"Enzar" (three times crystallized and purified trysin, Lot No. R550-13A) kindly supplied by the Armour Laboratory Research Department. They state that it is dialyzed salt-free and does not exhibit chymotryptic, ribonuclease, or desoxyribonuclease activity. The method of preparation should preclude the presence of amylases, lipases, and carboxypeptidase, but it is possible that some peptidases may be present.

collagen is provided in the present study. One-quarter of the total cases examined exhibited fibril changes identical with those produced by the action of collagenase and trypsin[4] (Table 3 and Fig. 1). These changes were far less frequent than those found even when using very low concentrations of collagenase, and probably would not have been noticed except for the detailed search at high magnifications required for this study.

Although the various pancreatic conditions included in the icteric, neoplastic and miscellaneous groups in Table 1 (as listed under Results) were not associated with any *quantitative* morphological alteration in the collagen of abdominal skin, a *qualitative* collagenase-like change was noted in seven of eight cases with jaundice, from whatever cause. The fibril ends recorded as "tapered" were either very long and sharply pointed or filamentous in type (Fig. 1). It is of possible significance that seven of the 28 cases listed in Table 3 as showing tactoids (short lengths of collagen fibrils tapered at both ends) and fibril narrowings also possessed filamentous fibril ends, and two of the individuals with the greatest number of fibril narrowings were both icteric.

Banfield's findings[1] of increased numbers of tapered ends where collagen is apparently being actively formed led to the hope that similar evidence of collagen activity might be demonstrated in diseases of the connective tissue and prompted the present study. However, this was based on the assumption that collagen throughout the body reacted as a single target organ and was equally affected by any possible collagenase- anti-collagenase imbalance. The methods used in this investigation were especially designed to keep the collagen unaltered and as near as possible to the condition found *in vivo*. The results show that abdominal skin collagen does *not* reflect the state of the collagen in other anatomical areas, the morphological appearance and number of tapered ends being the same in individuals suffering from both acute and chronic connective tissue disease and in those dying from a variety of other causes. Similarly, there was no difference with age. This does not in any way preclude enzymatic imbalance as a cause of collagen disease, as the areas containing the largest amount of collagen (synovial membrane, endocardium, tendons, etc.) plus mechanical wear and tear (joints, heart valves, and tendons over bony prominences) might be expected to receive the brunt of attack by any available uninhibited collagenase (perhaps hastened by local chemical alterations, change in tissue fluid pH, etc.), whereas the rarely involved abdominal skin might never show any change. Also the use of tapered ends as a morphological "index" of collagen activity may not be sufficiently sensitive to reflect alterations in collagen and/or ground substance metabolism, or in a possible soluble collagen precursor.

It was interesting that a similar percentage of tapered ends and fibril narrowings was found in the papillary layer of the dermis (attached to the epidermis) of two cases. This confirms Banfield's findings[1] that tapered ends are not strictly limited to one anatomical area of the body.

SUMMARY

The collagen from the abdominal skin of 104 individuals with and without so-called "collagen disease" was examined under the electron microscope, and the number of tapered fibril ends was determined and compared with the number of square or torn ends found in the same sample. At least 400 non-tapered ends were counted on duplicate grids prepared from each specimen, a total of 39,914 non-tapered ends being recorded on the adding machine. No significant difference in the percentage of tapered ends was found with age, type of disease, disease activity or therapy, all readings falling between 1 and 8%.

One-quarter of the individuals exhibited minimal changes identical with those found following the action of collagenase and trypsin on collagen *in vitro*. This suggests the possible presence of some substance in the dermis of people of all ages capable of altering collagen. Very long filamentous fibril ends were found in most cases of jaundice (from whatever cause) and seven others, of whom five had disorders of the connective tissue.

ACKNOWLEDGMENT

I am very grateful to the late Dr. H. Bunting, Drs. W. G. Banfield, and W. H. Gaylord for constructive criticism and guidance in this work. I am indebted to Dr. A. A. Liebow and the staff of the Pathology Department at the Grace-New Haven Community Hospital, Dr. B. H. Landing at The Children's Medical Center, Boston, Dr. R. Tennant of Hartford Hospital, and Dr. L. P. Hastings of St. Francis Hospital, Hartford, for autopsy material. I wish to thank Dr. P. B. Beeson and Dr. G. K. deForest of New Haven and Dr. J. C. Leonard of Hartford for permission to perform biopsies on adult patients, and Dr. J. R. Paul in whose department this work was done.

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EVENTS

WOODWARD LECTURE

April 5, 1954

PROVIRUSES IN THEIR RELATIONS TO VIRUSES AND CELLS. By Andre Lwoff, Chief, Department of Microbial Physiology, Pasteur Institute, Paris, France.

Lysogenic bacteria possess the hereditary power to produce phage. This power is maintained in the form of a specific non-infectious structure, the prophage, which behaves as a potentially lethal gene. Prophage should be considered as the genetic material of the phage anchored at a specific locus of a bacterial chromosome. The formation of phage from prophage may be induced at will by use of mutagenic-carcinogenic agents in some "inducable" lysogenic systems.

The various aspects of the relations between phage, prophage, and the bacteria have been discussed.[1] In defective lysogenic strains the disease induced by ultraviolet light does not culminate in virus particles. In bacteriocinogenic bacteria, the induced disease is the synthesis of a protein and it is this synthesis which is lethal. This process may be considered as a transition between metabolic and infectious diseases.

G. A. F.

PHARMACOLOGY-BIOCHEMISTRY SEMINAR

April 20, 1954

OXIDATIVE METABOLISM OF THE SULFUR AMINO ACIDS. By Thomas Singer, Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin.

Early phases of this investigation have shown that cysteinesulfinic acid, an assumed oxidative metabolic product of cysteine, could follow two routes of catabolism in *Proteus vulgaris*. One route leads to the eventual formation of pyruvate and sulfate, the other to cysteic acid.

The former reaction was shown to be effected through a transamination mechanism; thus, cysteinesulfinic acid reacts with α-ketoglutarate to yield β-sulfinyl pyruvate and glutamic acid. The β-sulfinyl pyruvate, catalyzed by Mn^{++}, then breaks down to pyruvate and sulfite non-enzymatically. It is suggested that the sulfite can be further oxidized to sulfate by an enzyme system such as the one described by Handler and his collaborators.

An analogous reaction was also observed. This reaction yielded aspartate and required a factor which was eluted from Dowex-1 columns through which cell-free *Proteus* extracts had been passed. The "Eluent Factor" was identified as succinic acid. Some question remains as to whether this compound is the true active component. It seems likely that the succinic is converted to fumaric, the fumaric to malic, and the malic to oxaloacetate; thus

[1] A. Lwoff, Bact. Rev. 1953, *17*, 269-339.

the oxaloacetate would be the other reactant. The reaction has been formulated as follows:

$$\text{cysteine sulfinic} + \text{"Eluent Factor"} + O_2 \xrightarrow{\text{DPN}} \text{aspartate} + \beta\text{-sulfinyl pyruvate}$$

$$\beta\text{-sulfinyl pyruvate} \xrightarrow{Mn^{++}} \text{pyruvate} + \text{sulfite.}$$

It is interesting to note that the transamination is accompanied by oxidation. This is the first reported instance of such a reaction.

An investigation of an animal system, rat liver mitochondrial powder, revealed essentially the same results as with the *Proteus* extracts when the cysteinesulfinic to pyruvate system was examined. No attempt has been made to characterize the cysteinesulfinic to cysteic acid reaction with the mitochondrial preparation.

MARY MYCEK

YALE MEDICAL SOCIETY

April 22, 1954

RECENT STUDIES ON PERMEABILITY AND INFLAMMATION. By A. A. Miles, Director of the Lister Institute for Medical Research, London.

The details of the organism's response to first infection, known as resistance or non-specific immunity, are poorly understood. Vascular phenomena, subdivided into amount of blood flow, blood pressure factors, and capillary permeability, are important, diminution of the first two by adrenaline and shock, respectively, leading to increased lesion size in guinea-pig skins injected with streptococci. The factor which produces increased capillary permeability is not definitely known. Histamine has a proven rôle in anaphylactic and other types of allergic reactions. We feel that leucotaxin, which has also received support as the important agent, produces its effect by functioning as a histamine activator, both substances having proportional effects when tested using guinea pigs injected intravenously with Evans blue dye; skin bluing gave evidence of increased capillary permeability. Although both these substances are inhibited by Neo-Antergan and other antihistaminics, some aspects of the inflammatory response are not affected by antihistaminics, and we believe that these substances have not been proven to be the general mechanism.

Capillary permeability induced by histamine and observed by the guinea pig skin-bluing method was found to persist for several minutes, following which the capillary became immune to further histamine action for as long as an hour, this effect also exhibiting cross-immunity with leucotaxin. We were able to demonstrate that this immunity was not due to vascular spasm or thrombosis. An alpha globulin with a gram-molecular weight of about 200,000 was identified in guinea-pig serum and later, in the serum of many other mammals. This substance, designated G2 alpha, has almost all of the

properties of histamine and 1.6x the activity. Its distribution in skin is not the same as that of histamine and it is not inhibited by antihistaminics. We believe G2 alpha to be an enzyme, perhaps a protease since only soya bean inhibitor was found to be effective in preventing its activity. We suggest that capillary permeability in inflammation may be due to the presence of G2 alpha which is then inactivated by a slowly-acting inhibiting factor, possibly an albumin.

M. C.

PHI DELTA EPSILON LECTURE

April 22, 1954

How Should the Non-psychiatric Physician Handle Psychiatric Problems in His Patient? By Robert P. Knight, Medical Director, Austin Riggs Center, Stockbridge, Massachusetts.

"Common sense," while a probable asset in a physician, is insufficient for his treatment of psychological problems. Every doctor's common sense is a collage of private factors and is not in the least related to the scientific uniformity toward which psychiatry strives.

The areas in which the general practitioner needs some knowledge of psychiatric principles are three: in everyday encounters with his patients, in the administration of minor psychiatry, and in the handling of major psychiatric problems.

Some principles which hold in general practice are:

1. Not thoughtlessly to harm the patient with your manner or your advice. Ill-considered comments or expressions by the physician in the course of an examination, before an operation, or just during "rounds," often create unnecessary and harmful anxiety in the patient. Harmful advice, such as bed-rest of too long a duration, has a decidedly detrimental effect on the patient's psychology and should be avoided.

2. Establish a good rapport with your patient. In sickness, the patient regresses psychologically. The doctor should realize this and use the regression to advantage in facilitating quick recovery. A pretentious bedside manner is an obstacle to establishing such communication. The romantic involvement which some women postulate for themselves with their doctor should be firmly and kindly discouraged.

A discussion of the treatment of minor psychiatric problems involves consideration of the doctor's time, temperament, and knowledge. In general, supportive therapy includes an examination of the conscious factors with the patient and the possible prescription of a change of environment or perhaps some medication, such as barbiturates for sleeplessness. Reassurance is often sufficient treatment.

Major psychiatric problems should be referred to a specialist, with neither embarrassment nor apology to the patient.

G. A. F.

POLIOMYELITIS STUDY UNIT

April 23, 1954

THE INTRACELLULAR DEVELOPMENT OF ANIMAL VIRUSES. By Igor Tamm, Rockefeller Institute for Medical Research, New York.

It is interesting to speculate on the possible ways in which the host cell may contribute to virus duplication by supplying the required energy and essential viral constituents. Recently, the knowledge of Vitamin B_{12}, and its importance in the metabolism of the single carbon fragments required for the synthesis of the purines and pyrimidines of nucleic acids, has been greatly enhanced. One wonders then, if analogues of Vitamin B_{12} might have inhibitory activity on the growth of viruses and cells and whether a study of this inhibition might shed some light on the biological specificity of viruses.

In these experiments, it was found that derivatives of benzimidazole produced varying amounts of inhibition of the growth of influenza virus, depending on the nature and position of the substituted groups on the benzimidazole nucleus; "2-5DMB (2-5 dimethyl benzimidazole), a polymethyl substitute, had fair inhibitory activity; but "DRB" (5,6 dichloro 1βD ribofuranosylbenzimidazole) had the highest inhibitory activity of all the substituted compounds. A comparison of "DRB" and "2-5DMB" revealed that "DRB" had a 35-fold greater effect on the inhibition of influenza B virus in membrane suspensions, and that this inhibitory effect was only partially reversible after withdrawal of the material. Further, none of the inhibitory effects could be blocked by the addition of Vitamin B_{12} or a number of similar analogues.

It is concluded, then, that the action of these agents is intracellular. The findings that "DRB" is able to inhibit virus growth at a concentration much lower than that which inhibits cell metabolism and that this inhibitory effect is not completely reversed on withdrawal would indicate that the main effect is taking place during the "latent period" of the virus.

WILLIAM POWELL

ALPHA OMEGA ALPHA LECTURE

April 26, 1954

ADRENAL CORTICAL FUNCTIONS IN STRESS. By George W. Thorn, Department of Medicine, Harvard Medical School, Boston, Massachusetts.

Since the time of Addison (1855) there has been much interest in the activities of the adrenal gland. Many physiological functions can be modified by the use of adrenal hormones, although these functions need not necessarily be primarily mediated by the adrenal hormones.

The degree of activity of the adrenal cortex can be correlated with eosinophile level of the peripheral blood, urinary output of 17-ketosteroids, or urinary output of 17-hydroxycorticoids. The use of urinary determinations of 17-ketosteroids in the male is relatively unreliable as an indication

of adrenal activity because the testes also elaborate these steroids. Experimentally, routine urinary methods employed account for only 30% of total steroids administered (50% at best). It is essential to remember that the fate of the remainder is unknown, and that interpretations based on the results of urinary steroid determinations may be subject to serious error.

By utilizing intravenous infusions of cortisone or hydrocortisone significant correlations between steroid blood levels and urinary steroid excretion can be made. Experiments indicate that there are three variables determining the response of the organism to ACTH: total dose of ACTH, duration of administration, and initial state of the adrenal gland.

Although epinephrine can cause a fall in eosinophiles, there is no evidence that this fall is in any way due to increased adrenocortical activity. There is, however, strong suggestive evidence that the effects of ACTH and epinephrine on eosinophile levels in the peripheral blood are additive. Exercise and cold, as types of organic stress, may be followed by a decreased eosinophile count but there is little, if any, reflection of adrenocortical activity in the urinary steroid output. Psychological stress produces a marked increase in 17-hydroxycorticoid excretion. A definite diurnal change has also been observed in the level of urinary 17-hydroxycorticoids. Surgical and psychological trauma or a combination of these most consistently produce adrenocortical activity as reflected by urinary steroid output. It should be emphasized that interpretation of such experiments must be made cautiously in view of the unknown fate of a large fraction of the body steroids.

J. M. Q.

HARRY BURR FERRIS MEMORIAL LECTURE

April 26, 1954

A Functional Interpretation of the Fine Structure of the Cytoplasm. George E. Palade, Rockefeller Institute for Medical Research, New York.

Under the electron microscope mitochondria are observed to have a complex structure, consisting of a double membrane, a homogeneous matrix, and a varying number of laminated, shelf-like structures, the *cristae mitochondriales,* protruding into the matrix. The cristae each have a membrane, approximately one protein molecular layer thick, and an inner space contained within the membrane. This space appears to communicate with the marginal space between the inner and limiting membranes of the mitochondria proper, so that enzymes, which are arranged, possibly, in assembly-line fashion along the crista membrane, are in a position to act on substrate absorbed through the outer, limiting membrane. In very motile or active cells there is a great number of cristae; these appear to be associated with primary energy production, cellular respiration. In striated muscle there is a functional localization of mitochondria, which here form double braces on each side of the significant contractile element, the Z-band. There are many

mitochondria at the axon end of synapses. In both cases the mitochondria may function as the main system for supplying energy to the active cells.

The endoplasmic reticulum, a system of tubules and vesicles, arranged more or less in a parallel manner and usually associated with a great profusion of patterned granules near the reticular locus, are especially prominent in glandular tissue. Microsomes seem to contain the enzymes used in protein synthesis; these structures also probably utilize the mitochondria as a source of energy. Capillary walls contain two rows of goblet-like vesicles—related perhaps to the endoplasmic reticulum—the vesicles of one row opening into the perivascular space, and those of the other row into the lumen of the capillary. These vesicles may be implicated, by actual migration, in the transport of material, both liquid and solid, across the endothelial wall. Such an interpretation is a speculative one, as are all these, but few people have as yet even seen the fine structures displayed in recent electron micrographs, let alone attempting to assign a function to them; this work is a pioneering effort.

J. S. PAGANO

ZOOLOGICAL JOURNAL CLUB

May 13, 1954

MORPHONGENETIC INFLUENCE OF THE EMBRYONIC SPINAL CORD IN THE AXIAL MESODERM. By Howard Holtzer, Department of Anatomy, School of Medicine, University of Pennsylvania, Philadelphia.

The reduction of the diameter of the neural tube of a late salamander embryo was followed by a reduction in the size of the cartilaginous canal about it. Conversely, by transplanting anterior sections of the tube to the posterior region of the animal, thereby effectively increasing the tube's diameter in the posterior region, an increase in the canal resulted. The removal of the neural canal altogether led to the formation of an amorphous cartilaginous rod. Furthermore, by inverting the tube, a normal but inverted canal would be formed. Thus, we conclude that the neural tube plays some rôle in determining the shape of the cartilage about it. Since this cartilage and the mesoderm from which it arises are not in direct contact with the neural tissue, subtle biochemical influences must be at work.

The notochord, on the other hand, can be shown to have inhibitory prowess—its removal leads to the premature formation of a centrum, which, in the salamander, normally does not appear until after the completion of the vertebral arches. It is of interest to note that in the human, where the centrum appears first, there is virtually no notochord present at the time of vertebra formation. In the regenerating tail of a salamander, too, where there is no notochord, the centrum develops first.

That the cartilage is not exclusively formed from the sclerotomes was shown by experiments in which various portions of the somites were extirpated. Wherever deficiencies occurred, they were muscle deficiencies. Even when 75% of the medial somite (sclerotome) was removed, the carti-

laginous arch was normal or near normal. Thus, since the cartilage-muscle ratio does not remain constant, we conclude that the regulative mechanism is probably not intrinsic to the somite.

When the neural tube is removed from a somewhat earlier embryo (stage 25), no cartilaginous canal will develop. However, a canal will develop about a piece of neural tube grafted under the skin, even though the graft is only in contact with lateral somatic mesoderm (myotome).

Studies of *in vitro* mouse tissue indicated that cartilage can be induced to form by the introduction of spinal cord, even though the latter is not in contact with the cartilage-forming cells. The somatic cells can be induced to do this only within a very specific time period. Nor were other tissues, with the exception of the cerebral cortex and otic capsule, found to have this chondrogenic effect. Presumably, too, the transmission of the chondrogenic substance is by means of direct transmission from somite cell, rather than by radiative diffusion.

P. K.

YALE MEDICAL SOCIETY

May 10, 1954

Presentation of M.D. Theses by Senior Medical Students.

THE EFFECTS OF THE INTRAVENOUS ADMINISTRATION OF ACTH AND HYDROCORTISONE UPON THE ELECTROENCEPHALOGRAM OF EPILEPTICS AND NORMALS. By Donald S. Kornfeld.

The observations of patients demonstrating hypo- or hyper- adrenal states reveal abnormalities in the central nervous system manifested both in psychic disturbances and EEG variations, which dramatically demonstrate the relationship of adrenal steroids and the human nervous system. Controlled studies to determine the effects of ACTH and the adrenal steroids on the central nervous system of laboratory animals have confirmed the clinical impression that these agents are neuractive. More recently ACTH and cortisone when used in therapy have been noted to produce seizures and EEG changes.

Fourteen subjects were utilized in this study, 9 epileptics and 5 controls. EEG records were taken with standard Grass equipment, utilizing 16 leads. Infusions were administered over a four-hour period. EEG recordings were made at the onset, at two hours, and finally at four hours, as the infusions were completed. Twenty-five g. of ACTH were administered in 500 cc. of saline to 4 epileptics and 3 controls; 100 mg. of hydrocortisone were infused in a solution of 5% glucose and 1% ethyl alcohol to 5 epileptics and 2 controls. The EEG records were studied by frequency spectrum analysis (Engel and Romano) and complete visual inspection. Eosinophile counts were used as a measure of steroid activity, while serum and urine samples of the 7 hydrocortisone subjects were analyzed for Na, K, and Cl.

Both ACTH and hydrocortisone would appear to have the ability to produce an increase in 5-7 cycle per second activity as detected by inspec-

tion analysis, although hydrocortisone would seem to exert this effort more consistently and to a greater degree than seen with ACTH. Two patients in the hydrocortisone series also manifested an increased incidence of 1½-2 cps. seizure discharges. Spectrum analysis, on the other hand, revealed a shift toward slower frequencies within the alpha range in 4 of the 7 subjects in the ACTH group. However, little change was noted in the hydrocortisone series.

In general, epileptics seemed more sensitive to the effects of these two drugs and the more abnormal records appeared more susceptible to change. A classical eosinopenic response was obtained in all but one of the patients studied. The one exception was a patient in the ACTH series who had been receiving cortisone therapy for a year. However, this patient demonstrated obvious slowing in her EEG which suggested that ACTH might very well have exerted a direct neurotropic effect. Hydrocortisone produced essentially no change in serum Na or Cl concentrations but did produce a significant elevation of serum K concentration in 5 of 7 subjects studied. The nature or degree of change noted in the EEG, however, was quite independent of the degree of change in K concentration.

DONALD KORNFELD

THE CORRECTION OF HYPOKALEMIC ALKALOSIS WITH POTASSIUM BICARBONATE. By Martin B. Vita.

Earlier investigators have shown that hypokalemic alkalosis can be corrected by potassium chloride. The experiment presented here was designed to determine the effect of potassium in the absence of chloride on such an alkalosis.

A group of 42 rats were made potassium deficient in three weeks by daily injections of DOCA; a diet lacking in sodium, potassium, and chloride; and an isotonic drinking solution having a molecular sodium to chloride ratio of 1.5/1.0. The DOCA injections were then discontinued, the drinking solutions replaced by distilled water, and the animals treated for a period of five days with intraperitoneal injections of a total of 30mEq. $KHCO_3$ per kilogram body weight. By sacrificing groups of these animals each day, it was demonstrated that all the serum and muscle changes of the hypokalemic alkalosis gradually returned to normal.

As there was no loss of muscle chloride during this correction, it was necessary to conclude that the rise in serum chloride noted was the result of reduction of extracellular volume while maintaining total body chloride constant. The detection of a marked increase in sodium and water excretion during correction with a concomitant diminution of urinary chloride confirmed this interpretation. A fact of considerable interest was the increasing disparity between the amount of base and chloride in the urine without increase in urinary bicarbonate to preserve electrical neutrality. The rise in bicarbonate necessary for such an effect would have resulted in urine pH changes far outside the physiological range. It became evident that the kidney had other means for the disposal of large amounts of base without

producing severe pH changes. As the daily output of organic anion in the urines of these animals quadrupled during correction, the mechanism appeared to be the substitution of organic acid for chloride.

Citric acid and alpha-keto-glutaric acid were shown to be the principal acids involved in this mechanism. There were other acids present in smaller amounts but were not identified.

Two general conclusions may be drawn from this experiment. First, the ratio of sodium to chloride being resorbed by the kidney may be altered by the potassium ion. Second, the volume of the extracellular phase as well as its electrolyte composition may be influenced by potassium intake.

MARTIN VITA

BOOK REVIEWS

ATLAS OF EXFOLIATIVE CYTOLOGY. By George N. Papanicolaou. Cambridge, Mass., published for the Commonwealth Fund by Harvard University Press, 1954. $18.00.

The *Atlas of Exfoliative Cytology* is a valuable distillate of the vast experience of the master cytologist, Dr. George N. Papanicolaou. The doubtless heavy subsidy from the Commonwealth Fund has been well employed. The book has been prepared in all modesty in loose-leaf form "to keep abreast of new advances," and an appeal is made for new material.

As befits an atlas, the illustrations are many and the text is brief but informative. Following a historical introduction, and a useful section on methods, there are general chapters devoted to each of the large subjects of the atlas—female genital system, urinary and male genital systems, respiratory tract, digestive system, exudates, and breast.

One bit of philosophy might perhaps have been discussed: that the initial study of the smears should be without knowledge of any aspect of the clinical history. How else can one avoid seeing with the eye of faith? How else can the contribution of the cytological method be evaluated quantitatively? The author seems to differ from this point of view and tends rather to stress the sources of error, for example, previous instrumentation or radiation and the need of a knowledge of these things in arriving at a final conclusion.

In the body of the volume each of the magnificent plates is accompanied not only by a caption for every illustration but also by a discussion. There is some repetition in these discussions of material presented in the general chapters, but the proximity to the figures perhaps makes this desirable. Sometimes the discussion adds nothing to the caption. Occasionally, the caption leaves the reader somewhat in doubt as to the true nature of the cells illustrated, as for example that which accompanies Figure 10 on Plate E II. It would be desirable, also, to know just what is meant by "early" carcinoma.

One cannot quarrel with the author's statement that the drawings cannot be compared with photographs. The latter are magnificent, there being some loss of verisimilitude only because the background has been removed. The warrant for use of the drawings is stated to be the difficulty of showing all levels of a multistratified cell mass. Yet most of the drawings show single cells or groups of isolated cells. The criticism is not of the artist, who has done superlatively well with a very difficult subject, but rather that the photographs have a closer resemblance to the actual material.

An atlas such as this is not intended as a "key" in the taxonomic sense. But it is a thing of beauty and wisdom valuable in extending the knowledge of those with less experience than its author; and who has more?

AVERILL A. LIEBOW

EPIDEMIOLOGY AND CONTROL OF EPIDEMIC SYPHILIS. By E. I. Grin. Geneva, World Health Organization, 1953. 93 pp. $1.00.

In June of 1943, Mahoney, Arnold, and Harris announced their epoch-making discovery that penicillin produced prompt regression of early, active

syphilitic lesions of man and rabbit. Within a very short time their observations were confirmed and extended, and the effectiveness of penicillin in the treatment not only of syphilis, but of other spirochetal diseases as well, was amply demonstrated. The biological studies of Fleming and the chemical investigations of Abraham and Chain and Florey had culminated in a therapeutic triumph over a disease which for more than four centuries had been a scourge of man. Five years after Mahoney's report, the World Health Organization of the United Nations adopted a Constitution which came into force on April 7, 1948; thus was realized years of effort to establish a single, inter-governmental, international health agency. These two grand achievements, one in the biological sphere, the other in the politico-social realm, joined forces to aid in one of the first systematic attempts to eradicate an infectious endemic disease through mass treatment. The disease is endemic syphilis, the treatment is penicillin, and the locale is Bosnia. The scope and conduct of the project are vividly described in the present volume.

Dr. E. I. Grin, the author of this report, served as Field Director of the program. In addition to other distinctions, he is Director of Health of Sarajevo, the principle city of the People's Republic of Bosnia and Herzegovinia in Yugoslavia. In clear and succinct prose, supplemented by numerous charts, tables, graphs, and photographs, he describes the natural course and epidemiology of endemic syphilis and gives a detailed account of the historical background of the disease. There are included also descriptions of the clinical aspects of endemic syphilis, its mode of spread, and the associated serological changes. The report is documented by a carefully selected bibliography. An Annex by Turner and Hollander, who studied two strains of treponemes isolated in Bosnia from patients with active lesions of endemic syphilis, concludes that the organisms belong to the general group of strains commonly designated *T. pallidum*.

Endemic syphilis occurs widely throughout Bosnia and Herzegovinia. The disease is most frequently acquired in childhood and is rarely transmitted through sexual contact. Poor social and economic conditions, inadequate sanitation, and crowded dwelling places all contribute to its spread. This occurs usually through the use of common domestic and household utensils, as for example the "ibrik" or water jug with its delicate curved tubular spout which the drinker puts to his lips. Early infectious lesions often occur in the oral region, but primary lesions are relatively infrequent, having been observed in less than one per cent of the cases. Contrary to early beliefs, cardiovascular and neurological involvement do occur. The rarity of congenital infection in endemic syphilis is explained as resulting from the lapse of many years between childhood infection and maturity, and the known fact that infectiousness in syphilis decreases with the passage of time.

The organization of the field campaign is described, with emphasis on the case-finding techniques and the serological screening procedures. All clinically detected cases of syphilis were treated immediately. Treatment was also given to all persons with positive serological reactions. About 95 per cent of the total census population in the endemic areas was examined, and 35,238 cases were treated in the campaign which began in 1948 and con-

tinued for several years. Procaine penicillin G in oil with 2 per cent aluminum monostearate (PAM) was employed. For adults the schedule was 3.6 to 4.2 million units given in six or seven injections of 0.6 million units daily or every second day. In some series the total dose was given in a single injection with no difference in end-result. Retreatment for serological or clinical relapse usually duplicated the original schedule. Treatment results were decisive. No case of early endemic syphilis failed to respond, and gummatous lesions were rapidly arrested. Abortive treatment of family contacts —persons with negative clinical and serological findings who had been exposed to infection from other members of the family—effectively reduced the reservoir of infection. The abortive treatment schedule was a single injection of 1.2 million units of PAM. Frequent follow-up examinations resulted in the discovery and treatment of new cases previously not treated, and of individuals with reinfections or relapses. The author concludes that it is possible under favorable conditions and in limited areas to eradicate the infectious reservoir completely. In other areas the disease can be checked, the infectious reservoir reduced to a minimum, and ultimately completely eliminated.

This study is the first of a series on treponematoses, prepared under the auspices of the World Health Organization. It establishes an admirable precedent which it is confidently expected will be emulated in subsequent publications.

PAUL D. ROSAHN

SHOCK AND CIRCULATORY HOMEOSTASIS. Transactions of the Second Conference sponsored by the Josiah Macy, Jr. Foundation, October 19, 20, and 21, 1952. Harold D. Green, Ed. New York, 1953. 275 pp. $3.75.

This record of the Second Conference on Shock and Circulatory Homeostasis of the Josiah Macy, Jr. Foundation, held in October, 1952, contains detailed information on two main subjects related to the problem of shock: (1) the metabolic aspects of hemorrhage and traumatic shock, presented by Drs. Frank L. Engel, Benjamin W. Zweifach, Ephraim Shorr, and Jacob Fine, and (2) the distribution and possible physiological functions of epinephrine, presented by Drs. U. S. von Euler, Raymond P. Ahlquist, Ewald E. Selkurt, Harold D. Green, and G. C. Cotzias. Merging almost indistinguishably with the opening presentations of data by the above authors are the free and revealing discussions by the many other participating members of the conference, all of whom are well-known workers in the field.

The result is a multi-disciplined approach to such varied topics as the impaired carbohydrate utilization, negative nitrogen balance, and ketosis of shock; the rôle of the adrenals, of VDM and VEM, of hepatic circulatory deficiency and infection, and of various autonomic blocking agents in the irreversibility of shock; the formation, function, and differentiation of epinephrine and norepinephrine under normal and pathologic conditions. An interesting introduction is included in which more or less detailed auto-

biographies give the reader a more intimate knowledge of the interests and research careers of each of the participants.

Because the book is a running account of the provocative discussion of detailed experimental data with extensive bibliographical documentation, it is of exceptional interest to all workers in the fields related to normal and pathological cardiovascular physiology. Although there is a good index, the two main sections of the book are best read (for the first time, at least) as a continuous whole, rather than for reference purposes.

ALLAN V. N. GOODYER

NOBEL PRIZE WINNERS IN MEDICINE AND PHYSIOLOGY 1901-1950. By Lloyd G. Stevenson. New York, Abelard Press, Inc., 1954. ix + 291 pp. $6.50.

Dr. Lloyd G. Stevenson has compiled the Nobel Prize Winners' own description of their work, usually taken from the Nobel Lecture delivered when the prize was presented. To accompany these the author, associate professor of medical history at the University of Western Ontario, has included short biographical sketches plus longer sections interpreting the scientists' achievements as seen now. These last, "Consequences in Theory and Practice," are good, and the discussion of Sir Charles Sherrington is one of several that are excellent.

Unfortunately, this book, which is part of a series on Scientific Nobel Prize Winners published by Abelard Press, allots each scientist exactly five pages and no more. The biographies are so brief, therefore, that they seem to be merely a one-paragraph listing of dates and professorships. Some of the quotations from the laureates also suffer from frequent deletions.

Since there is so little personal information about the scientists, this book will seem uninteresting to many readers. As a reference volume, however, containing concise explanations and well-chosen quotations from the award winners, this book is recommended for scientific libraries.

GEORGE PAULSON

AN OUTLINE OF GENERAL PHYSIOLOGY. By L. V. Heilbrunn. 3d ed. xiii + 818 pp. Philadelphia and London, W. B. Saunders Co., 1952. $10.50.

Although the scope of this popular work has not been changed, it has undergone considerable revision, in part reflected by the bibliography, more than one-third of which includes new references. Preserved and even strengthened is the author's conviction that a variety of biological phenomena can be explained in terms of colloid chemistry. In his words, "the protoplasm of cells is always subject to two sets of influences, those that tend to clot it and those that tend to prevent this clotting." The responses of protoplasm to stimulation are believed to be due to a sensitivity to free calcium ion, which is released from the cortex of the cell and which "then produces a clotting of the interior of the cell, a clotting similar to the clotting of blood." Thus, "the injurious effects of cold and heat, the response to stimulating agents, and the impetus to cell division can all be integrated in terms of

calcium release." Integrated in this way are permeability, contraction, conduction, the action potential, mitosis, shock; the effects of ions, anesthetics, other drugs, and radiation are also explained in terms of protoplasmic clotting. An extensive bibliography—including many references to the work of the author and his students—is marshalled both to fortify his own theory and to present and examine others.

J. P. G.

THE MIND AND THE EYE. By Agnes Arber. New York, Cambridge University Press, 1954. xi + 146 pp. $3.00.

"Natural science, though beginning with material phenomenon, is transformed, finally, into natural philosophy. It is to this synthetic discipline that biology, in its autonomous aspect, belongs; its function is fulfilled when it offers its mite towards the ultimate fusion of metaphysical and scientific thinking." It is towards a justification of this conclusion that the author addresses herself in this volume. Beginning with a detailed consideration of the basic steps in biological research, she subsequently proceeds with an analysis of the bases of biological thought and their place in an over-all system of knowledge.

Mrs. Arber's choice of problem selection, accumulation of relevant data, interpretation, proof, communication of conclusions, and the final synthesis as the six basic steps will perturb no one. It is certain, however, that her penetrating analysis of these processes will come as something of a shock to those biologists—technicians might be a more appropriate term—accustomed to the assumption that the rigid tenets of pure logic are wholly applicable to biology. While true for its physio-chemical aspects, biology otherwise has more of the quality of a creative art. The rôle which intuition plays must be recognized. This is underscored by Einstein's opinion that our belief "that the regulations valid for the world of existence are rational is a faith springing from the sphere of religion."

This creative element in biology is further keynoted by the "underlying bias towards a belief in the Uniformity of Nature and hence in the unity of all things."

The thorough documentation and ample number of references cited on behalf of her work make it of particular value to scientists embarking on philosophical studies. There is indeed a relation between the seeing eye and the thinking mind, and this elucidation of that relation is an especially noteworthy one.

P. K.

THE BIOCHEMISTRY OF THE NUCLEIC ACIDS. By J. N. Davidson. 2d ed. New York, John Wiley & Sons, Inc., 1954. viii + 200 pp. $2.25.

The appearance of the 2d edition of this lucidly written little volume is necessitated by the rapid rate of progress in the nucleic acid field. As in the first edition, Professor Davidson has been able to weave the factual content

of his subject into a narrative which moves swiftly and retains the reader's interest to the last chapter.

The volume is similar to the first edition in form, but material now obsolete has been discarded and much new material has been introduced in an attempt to bring the book up to date. As the author states in the preface, this book does not claim to be a monograph which gives an exhaustive treatment of the biochemistry of the nucleic acids. Rather, it is intended to provide an elementary outline of the main features of the nucleic acids and nucleoproteins, and this objective is fully realized.

Among the topics covered are the chemistry, methods of analysis, and properties of the nucleic acids and polynucleotides. Several chapters are devoted to a discussion of enzymes involved in nucleic acid metabolism, the over-all metabolism of nucleic acids, the biosynthesis of nucleic acids, and the biological activity of nucleic acids, nucleotides, and nucleoproteins. All of this material is co-ordinated with a discussion of the cytochemistry of the nucleic acids. The net result of these varied discussions is a complete, well-integrated picture of a complex field which should be a boon to investigators in related disciplines who have not had the opportunity to keep abreast of this rapidly moving field.

As the author states in the preface, his book is directed to that class of readers who want an introduction to the subject, and for this group it is unusually well suited. The volume, while adequately documented, is not an encyclopedic collection of facts; accordingly, the person seeking a comprehensive work should turn elsewhere.

PETER S. CAMMARATA

THE DYNAMICS OF VIRUS AND RICKETTSIAL INFECTIONS. Frank W. Hartman, Frank L. Horsfall, and John G. Kidd, Eds. New York, The Blakiston Company, 1954. 461 pp. $7.50.

A practically verbatim acount of an international symposium held at the Henry Ford Hospital, Detroit, in October, 1953, this publication ought to share shelf space with *Viruses 1950, Advances in Virus Research,* the 1953 *Cold Spring Harbor Symposium,* Luria's *General Virology,* to mention a few recent books devoted mainly to the nature or "essence" of viruses; for the volume under consideration complements these in that for the most part it treats viruses from the standpoint of the host. Thus, the symposium was divided into five sections with respect to viral and rickettsial diseases: Mechanisms of Infection (12 papers), Ecology and Pathogenesis (6 papers), Mechanisms of Immunity (4 papers), Laboratory Diagnosis (5 papers), and Approaches to Prophylaxis and Therapy (6 papers). It was a pleasure to see that the contents of the papers actually fitted the section into which each was placed, for symposia are sometimes disconcerting to the extent that the logic governing the categorization of papers may be difficult to follow. In the present instance, as we have said, this is not the case. Ample room was provided for animal, plant, and bacterial viruses alike, especially in the first and second sections. Beyond that the presentations center about animal viruses and the rickettsiae except for some of the

discussions following each group of papers in which analogies and dissimilarities in the basic nature of the three classes of viruses are at times brought forth. The discussions are printed in their entirety.

The authors are all well known, if not famous, men in their fields, and the international flavor of the meeting was supplied by travellers from Australia, England, Malaya, and Denmark taking part in the readings. Some of the discussants came from other lands as well. The editors deserve warm praise for a forthright and beautiful compilation of this symposium—a worthy addition to viral and rickettsial literature.

LISBETH M. KRAFT

UNDERSTANDING THE JAPANESE MIND. By James Clark Moloney. New York, Philosophical Library, Inc., 1954. 252 pp. $3.50.

This book is an interesting admixture of psychoanalytic theory, cultural anthropology, historical reconstruction, and prophetic philosophy. Dr. Moloney subscribes to the plausible assumption that the psychodynamics of Japanese national character can be understood by integrating the facts known of Japanese culture, historical tradition, patterns of child-rearing, and Japanese distortions of imported ideologies, particularly psychoanalytic theory and practice. He proposes that these distortions are a function of cultural pressures that are also exerted on each Japanese individual throughout his life. These pressures originate in the Japanese authoritarian, oligarchical tradition and have as their goal the total conformity of the individual to the dictates of a highly crystallized hierarchical militaristic class tradition. In short, the Japanese are accused of having a "disindividualizing" culture.

Dr. Moloney focuses on the effects of Japanese disindividualization on the anger-rage-aggression manifestations of the Japanese individual and nation. He submits the hypothesis that the Japanese as a nation suffer from inordinate internalized hate and repressed rage. His evidence is an interpretative analysis of the crime rate (low, indicating repression of aggression against Japanese society), insanity rate (low, indicating "the Japanese emotional disease of neurotic conformism is an almost universal character neurosis which protects the Japanese from developing psychoses"), psychosomatic disease rate (high, indicating "bodily changes due to repressed rage"). The author maintains that only one outlet for aggression is permissible under the Japanese cultural-traditional system, namely, the explosive and periodic venting of accumulated pent-up hatred on targets external to territorial Japan. The "principle of coevality" dominates Japanese historical tradition and provides the moral justification and support for the extranational dispersal of Japanese hate. This principle of coevality is the mystical concept that Japan, consisting of a homogeneity of disindividualized persons, is without boundaries and is timeless, having no beginning nor end, and is coeval with heaven, earth, and the unlimited power and person of the Emperor.

This ancient national dedication to world domination is served by the imitative activities of the Japanese. The author presents numerous examples

of compulsive copying and syncretizing of foreign principles and practices with Japanese ways. He interprets this as indicating "identification with the enemy" and "neutralizing the threatening presence," so as to leave intact the potentiality of eventually establishing the Kingdom of Japan on Earth.

The syncretization of western psychoanalytic theory and practice with established Japanese cultural patterns receives special attention in this book. The author is convincing in his thesis that the liberalism and concept of freeing the individual from inner restrictions, characteristic of the goals of western psychoanalysis, are incompatible with Japanese culture, and that the Japanese psychoanalytic movement exists today only because those goals have been changed so as to reinforce the individual's inner restrictions and permit increased conformity rather than increased freedom.

This book is timely, the author prophetic, and his message ominous. He warns that it is politically naïve to assume that the Japanese have integrated democratic ways of life foreign to their culture and tradition. He intimates that sooner or later Japan will again revert to war against the "enemy," i.e., extraterritorial Japan: "If the Perry-Harris sneak attack on Japan led to Pearl Harbor, what might we eventually expect in return from the desolation of Hiroshima by our first atom bomb?"

The book is vulnerable to some criticisms. The style, lively at the onset, becomes repetitious and eventually tiresome. Forty-five pages are devoted to abstracts from translated Japanese psychoanalytic writings, presented, it would seem, with the implication that Dr. Moloney believes most Japanese analysts should turn in their couches. One senses that Dr. Moloney is anxious in his rôle of prophet who, looking at Japan's past and present with psychoanalytic insights, predicts a fearful future. Perhaps his anxiety is responsible for the dogmatism that characterizes the many sweeping assumptions and conclusions he makes on debatable issues such as the homogeneity of the Japanese people, the reliability of Japanese statistics, the etiology of schizophrenia and psychosomatic disorders, and the predictability of behavior of individuals and nations.

Dr. Moloney offers no constructive program for coping with the problem of the totalitarianism of Japan (or elsewhere). He does, however, contribute to our understanding of it and warns us to be on guard!

LOUIS B. FIERMAN

STREPTOCOCCAL INFECTIONS. Maclyn McCarty, Ed. New York, Columbia University Press, 1954. x + 219 pp. $5.00.

This book is an interesting compilation of fifteen papers read at the seventh symposium on microbiology held at the New York Academy of Medicine in February 1953—which indicates that this is neither a text nor a reference book in the usual sense. What may be less obvious is that it is not simply a group of original papers. The authors are authorities who explain through their special interests what is known and what is not known of the organism and host factors responsible for the varied manifestations of infection by hemolytic streptococci. Use is made of the investi-

gations of others but the bibliographies are not lengthy. The tables are clear and serve to stress points. The material is up to date. All combine to make the book a good starting point for one with propensities toward this field and specifically in rheumatic fever and nephritis.

Dr. Maclyn McCarty, the editor, states in the preface that a volume of this type has its inherent drawbacks, namely, lack of complete coverage of the subject-matter and continuity. It is my feeling that this may be a small loss in favor of a large gain to most readers. The book reminds me of a series of short stories by different authors as opposed to an exhaustive and exhausting tome. Editorial revision, which probably accounts for absence of serious repetition, does not alter the varied styles of the individual authors.

In summary, this book can be recommended to those with interest in hemolytic streptococcal illnesses and infectious diseases in general. A complex subject is handled in a manner that results in a smooth and accurate presentation of facts.

PAUL L. BOISVERT

1954 MEDICAL PROGRESS. Morris Fishbein, Ed. New York, The Blakiston Co., 1954. x + 345 pp. $5.00.

In this volume is undertaken the difficult task of cataloguing in summary form the advances in medicine over the last year. There are twenty chapters by various authors in specialized fields including cardiology, surgery, nutrition, gastro-enterology, rheumatic diseases, endocrinology, newer drugs, urology, infectious diseases, dermatology, ophthalmology, ear, nose and throat, allergy, gynecology, orthopedic surgery, psychiatry, rehabilitation medicine, medical psychology, and physical medicine.

Although a number of the chapters are simply expressions of opinion, ten of the reviews are well documented and Unger's review on allergy has 262 references. The subjects of antibiotic and steroid therapy are widely discussed. P. H. Long states that "physicians may look forward to the time (1964) when tuberculosis will no longer be a health problem of major importance. When this happens there will not be any diseases produced by bacteria which will be important as major causes of death in this country." However, Long emphasizes the development of resistant strains of staphylococci, and Unger emphasizes the increasing number of anaphylactic reactions to penicillin. Hurxtal presents a useful classification of syndromes responsive to cortisone and other steroids in his chapter on endocrinology; the syndromes are discussed as to etiology and as to course and prognosis. Mayo and Smith discuss adrenal cortical insufficiency in surgical cases where cortisone therapy has been recently received. A note of caution is injected into classification of diseases as "collagen" diseases by Boland in the chapter on Rheumatic Diseases. He points out that identical collagen changes have been found in chronic inflammatory reactions, myxedema, and certain tumors, as in disseminated lupus erythematosus, periarteritis nodosa, scleroderma, dermatomyositis, and malignant nephrosclerosis which presumably belong to the group.

This book is clearly intended for the general practitioner but it suffers from rather loose organization, the necessary cursory treatment of some subjects, and a limited index.

HARRIS BUSCH

ENCYCLOPEDIA OF ABERRATIONS. Edward Podolsky, Ed. New York, Philosophical Libraries, Inc., 1953. vii + 550 pp. $10.00.

This book contains a wealth of material on a large number of psychiatric topics arranged in alphabetic order. Most of the treatises, and especially the best ones, are reprints of articles published by the respective authors in various journals and the latter often represent authoritative thinking in the particular fields. There are, however, glaring omissions, which are aggravated by a very unusual choice and arrangement of terms, rendering this book rather useless as an encyclopedia. For instance, under addiction one finds a brief and very inadequate statement concerning the dynamics thereof but no cross-reference is given to the many other papers in the book concerning various types of addiction, some of them excellent in themselves. Therefore, finding them depends entirely upon the ingenuity of the reader in guessing what term the editor chose for the particular condition (there is, of course, no index). To illustrate further, under alcoholism one finds a paper on "chronic" (without cross-reference to addiction again) covering fourteen pages, and then ten pages are devoted to a reprint on etiology presenting a very doubtful and controversial viewpoint. By implication then, disturbed behavior engendered by the excessive intake of alcohol in the acute form, presumably one of the most commonly encountered "aberrations," is not considered such. Despite the authors' great predilection for Greek and other words of foreign derivation, the word dipsomania does not appear. Along the same line "masturbation" is not listed but is found under "onanism."

The most severe criticism pertains to the ambitious title. First of all, the book is not "encyclopedic" (complete or encircling knowledge) concerning aberrations, if only because it does not state what type of aberration the book is to be about. Even if delineation of the type of "aberration" was omitted for the sake of brevity the confusion in the conception of the book is revealed by the fly-leaf which speaks of aberrations in behavior, whereas the foreword calls it "a comprehensive study in the ever-widening field of psychopathology." In brief, this book represents an aberration in encyclopedias rather than an encyclopedia of aberrations.

STEPHEN FLECK

THE JEALOUS CHILD. By Edward Podolsky. New York, Philosophical Library, Inc., 1954. xii + 147 pp. $3.75.

In this book the author presents his concept of the importance of jealousy in the life of the child. His chapter headings illustrate the large area he has attempted to encompass. He touches on the psychological problems of the

child with physical defects, rheumatic fever, tuberculosis, diabetes, obesity, glandular disturbances, and speech disorders. There are chapters on problems of children who are adopted, are illegitimate, or who belong to minority groups.

There are sections and paragraphs which are well written and accurate. However, the book as a whole, in the opinion of this reviewer, is not likely to increase the knowledge or understanding of children by the parents, teachers, social workers, or psychologists to whom it is directed. The attempt to condense and summarize so many important concepts has caused the writer to oversimplify to the point of misinterpretation and confusion. That the reviewer frankly takes issue with many of the author's formulations is of less importance than that what he does present is not clearly developed, and that the advice on how to handle problems is in the oversimplified "recipe" form which is so seldom of help either to parent or to professional workers in the field.

SALLY PROVENCE

FLUID AND ELECTROLYTE THERAPY. By Franklin L. Ashley and Horace G. Love. Philadelphia, J. B. Lippincott Co., 1954. 72 pp. $3.00.

This book represents another attempt to provide a short guide to fluid therapy. Like others which the reviewer has seen, the discussion is largely concerned with certain rules of thumb which may have been successful in the author's hands. Yet such is the prestige of books that the reader may believe that the fundamental principles by which modern therapy is applied are described. Fluid therapy cannot be described in fifty-eight pages so that a doctor can apply modern knowledge to the various problems.

To a pediatrician, the summary dismissal of the physiological basis of metabolism of water and electrolyte by doctors treating adults is hard to understand. Yet the fundamental physiology is the same in adults and infants. The confusion of the surgeon who attempts to prescribe fluid therapy for infants and children will not disappear until all fluid therapy is based on principles applicable to patients of all ages and body sizes. The principles are relatively simple: (1) Water expenditure is normally related to metabolic turnover and the most convenient unit of reference is caloric production; (2) deficits of water and electrolyte are related to body size and for the most part the differences in composition of infants and adults do not lead to gross errors of estimation; (3) rates of abnormal expenditure are related to both metabolic turnover and the type of physiological disturbance. No rules of thumb can ignore these facts without leading to inappropriate therapy in many cases. Furthermore, difficult cases can best be analysed in these terms.

The reviewer does not believe that KCl or $CaCl_2$ should be injected until symptoms are relieved. Diagnosis is too likely to be erroneous for dosage to be judged by clinical improvement. The discussion of metabolic alkalosis is incomplete. The rôle of intracellular changes in composition is hardly discussed.

The reviewer does not believe that fluid therapy can be made simple, but he does believe that the subject is no more difficult than many which a doctor does learn. To meet the needs of the doctor who wishes to apply modern knowledge, a book must present the difficulties in physiological terms. The subject only remains incomprehensible when an attempt is made to avoid the fundamental difficulties by developing a system of rules of thumb.

DANIEL C. DARROW

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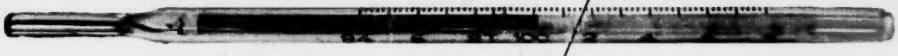
"Symptoms, including fever,

largely cleared up

within 24 to



48 hours."

English, A. R., et al.:
Antibiotics Annual (1953-1954),
New York, Medical
Encyclopedia, Inc., 1953, p. 70.

AIH

afebrile in hours c

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